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OCCURRENCE OF FREE γ-AMINOBUTYRIC ACID IN BRAIN AND ITS FORMATION FROM L-GLUTAMIC ACID

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The free amino acids of tissues represent an important fraction of the nitrogenous components of the living cell. Their distribution has been studied by Van Slyke (1913) using the nitrous acid method of analysis. However, only the entire group could be measured and an index of their total concentration established. With the advent of partition chromatography on filter paper (Consden, Gordon, and Martin, 1944) new strides have been made in the analyses of free amino acids in tissues. In our laboratory we have surveyed the free amino acid spectrum of many organs of the rat (Awapara, Landua, and Fuerst, 1950), some human tissues and tumors. In the course of this survey we found several components which differ chromatographically from any of the better known amino acids. Among such components, we identified a substance which was present in nearly all tissues examined. This substance was isolated from beef spleen and identified as aminoethylphosphoric ester (Awapara, Landua, and Fuerst, 1950). A second unknown fraction was detected in the brain of rats, and later on it was found in human brain, guinea pig, rabbit, pigeon, and beef. The chromatographic behavior of this component indicated that it may be γ-aminobutyric acid (Figure 1). It possessed identical retardation coefficient as γ-aminobutyric acid in several solvents. The unknown material has been isolated from beef brain and identified (Awapara, Landua, Fuerst, and Seale, 1950).

METHODS

For the isolation of γ-aminobutyric acid, extracts were prepared from brain. Proteins were removed by thermal coagulation; peptides and other substances were removed by alcohol precipitation. An alcoholic extract was thus obtained which

*From the M. D. Anderson Hospital for Cancer Research, Houston. Presented at the Cancer Symposium May 12, 1950.

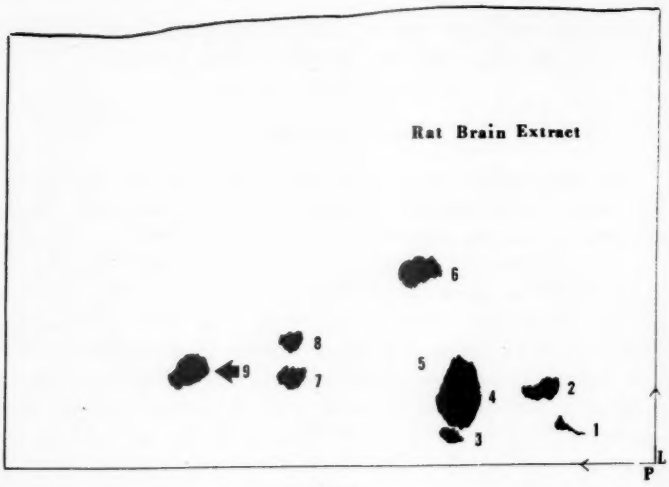

FIG. 1

Chromatogram of protein-free brain extract. The following components are discernible: (1) Glutathione, (2) Aspartic acid, (3) Aminoethylphosphoric ester, (4) Glutamic acid, (5) Glycine, (6) Taurine, (7) Glutamine, (8) Alanine, (9) δ-aminobutyric acid.

contained all the free amino acids, lipids and other alcohol soluble materials. Lipids were removed by the simple device of treating the extract with chloroform. A two phase system is formed, and the amino acids distribute themselves between the chloroform-alcohol layer and the water layer, the aqueous layer containing the bulk of polar substances including all the free amino acids. This aqueous extract was used for chromatography on starch (Stein and Moor, 1948). The method used was identical to that described by Reed (1950). Thus a pure fraction of γ-aminobutyric acid was obtained which was used for the preparation of a silver salt. Analyses of the silver salt gave indications that the substance in question was an aminobutyric acid. Proof of identity was furnished by paper chromatography of the unknown mixed with all the possible aminobutyric acids. Only γ-aminobutyric acid gave a single homogeneous spot. All others were resolved by the procedure.

Approximate quantitation of the γ-aminobutyric acid in brain was accomplished by the method of Landua and Awapara (1949).

Discussion

The occurrence of free γ-aminobutyric acid in animals has been reported before by Dent (1948), who found it, in only trace amounts in blood and urine. It has been known for some time that in microorganisms and plants, glutamic acid is decarboxylated and γ-aminobutyric acid produced in the process. Gale (1940) studied the glutamic acid decarboxylase in various strains of bacteria and in some cases discovered that the process was quantitative in a short period of time. Krebs made use of this reaction and developed a manometric method for the estimation of glutamic acid. Many plants have been investigated and finding glutamic decarboxylase is not uncommon.

It is surprising, however, to find such large concentration of free γ-aminobutyric acid in brain. It has been known for some time that glutamic acid is the only amino acid which maintains respiration of brain slices (Krebs, 1935; Weil-Malherbe, 1946). The mechanism is an obvious oxidative deamination. The presence of an enzyme capable of decarboxylating L-glutamic acid was investigated in rat brain. Using homogenates it was possible to demonstrate the conversion of glutamic acid into γ-aminobutyric acid by means of manometry and partition chromatography on filter paper (Wingo and Awapara, 1950). This conversion has been also demonstrated by Roberts (personal communication) using radioactive glutamic acid.

The importance of this reaction is at present not known and the metabolic fate of γ-aminobutyric acid remains to be investigated. Glutamic acid is known to be absorbed readily from the blood stream by the kidney and liver but it may not penetrate the brain (Friedberg and Greenberg, 1947). The method used in the estimation has been the ninhydrin manometric procedure of Van Slyke. The method being specific for α-amino acids would fail to measure γ-aminobutyric acid which

could be formed rapidly from the injected glutamic acid. The end result would appear as if no absorption had occurred.

Examination of brain tumors has been difficult because of the obvious scarcity of material. One single case of brain tumor was studied and surprisingly enough we found no γ-aminobutyric acid. The absence of this compound was not confined to the tumor but also to the neighboring brain tissue which was supposed to be free of malignancy. Until a large number of brain tumors are investigated no explanation can be given to this finding.

Conclusion

The discovery of free γ-aminobutyric acid in brain has led to the discovery of a new enzyme which may play an important role in the metabolism of brain. It may partially explain the apparent sluggishness of brain in absorbing glutamic acid from the blood.

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SYNTHESIS OF QUATERNARY SALTS OF HETEROCYCLIC NITROGEN COMPOUNDS PRELIMINARY TO THE USE OF LABELED COMPOUNDS IN THE STUDY OF CANCER CHEMOTHERAPY

CARL T. BAHNER*

The first problem which confronts an investigator who wishes to learn something of the principles involved in cancer chemotherapy is to find compounds which destroy, damage, or at least inhibit the growth of cancer cells. The search for substances which will achieve these results is an ancient one (Woglom, 1947). It has been rewarded so often with disappointment rather than success that we are forewarned against any premature enthusiasm. However the first task before us is made somewhat less difficult by the fact that a substance need not be practical for use in treatment of human beings in order to throw some light upon the subject. Compounds which are very toxic or often produce harmful side effects can serve to help us learn how to attack the malignant cells.

Within recent years a number of compounds have been reported to cause necrosis of implanted sarcomas in mice. Among

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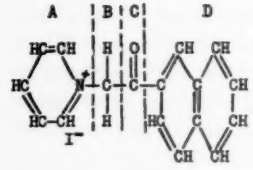
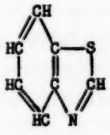
these are several quaternary salts of heterocyclic nitrogen compounds, such as β-naphthacylpyridinium iodide oxime (Hartwell 1947, Dalton 1947). In search of other active compounds and data which might aid in correlating structure with necrotizing action we have been synthesizing a variety of compounds which are in some sense analogs of β-naphthacylpyridium iodide or of phenacylquinolinium bromide. The plan of attack is based upon two complementary approaches: an extensive phase involving the synthesis of a wide variety of compounds, often quite different in some respects from the prototypes chosen, and an intensive phase involving a study and attempt to understand the effects of slight changes in the structure of compounds found to be active. The use of labeled compounds is expected to assist in elucidating the manner of action of the chemotherapeutically active substances obtained. A few of the compounds chosen have been prepared previously by other workers for other purposes and had not been tested for effects on malignant cells, but about 90% of them appear to be new substances.

A major line of attack has been to prepare compounds which contain two or more atoms other than carbon in a ring, instead of a pyridine ring. One of the first such series synthesized for this purpose consisted of salts containing a thiazole ring, in 4-methylthiazole, 2, 4-dimethylthiazole, 2-ethyl-4-methylthiazole, 4-methyl-5-β-hydroxyethylthiazole, benzothiazole and 2-methylbenzothiazole (Bahner, Pickens and Bales, 1948). The bases which were not available from commercial sources were synthesized by the method of Schwarz (Bachmann, 1945; Merck, 1939). Most of them formed crystalline salts upon heating five minutes at 100° with phenacyl halides, employing a small amount of alcohol as solvent when necessary to obtain a homogeneous mixture, but 2-chlorobenzothiazole, 2-methylmercaptobenzothiazole and 2-phenylbenzothiazole did not. The phenylethyl and cyclohexylethyl halides on the other hand reacted much more slowly, requiring three or four days at 100° in a sealed tube. In prospect these salts seemed especially interesting because of the kinship with thiamin, which is a quaternary salt of 4-methyl-5-β-hydroxethylthiazole, and because of the possibility of using a radioactive isotope of sulfur as a tracer, but none of the 21 compounds in this series on which

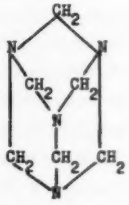


β-Naphthacylpyridinium iodide

Thiazole



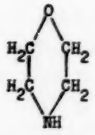
Benzothiazole



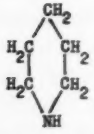
Hexamethylenetetramine

Pyrazine

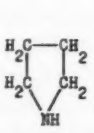
Quinoxaline



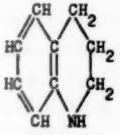
Morpholine



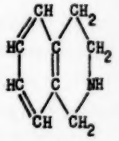
Piperidine



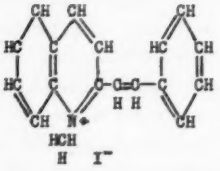
Pyrrolidine



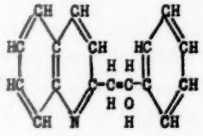
Tetrahydroquinoline



Tetrahydroisoquinoline



1-Methyl-2-styrylquinolinium iodide



1-Phenyl-2-(2-quinolyl)-ethanol

screening tests have been carried out have shown sufficient promise to justify further study.

More encouraging results have been obtained with a series of hexamethylenetetraminium salts (Bahner and Pickens, 1948; Bahner, Pickens, Pickens and Easley, 1950). Years ago Jacobs

and Heidelberger (1915) prepared a long series of quaternary salts of hexamethylenetetramine for study as bactericides (Jacobs, Heidelberger and Bull, 1916). Several of their preparations have been repeated and a number of new compounds of this type have been prepared. These white, crystalline salts are not readily soluble in cold water and tend to decompose on heating, but preliminary tests by Dr. C. E. Carter and associates, of Oak Ridge National Laboratories, using freshly prepared, very dilute, aqueous solutions of the phenacyl bromide, p-fluorophenacyl bromide, p-chlorophenacyl bromide, p-phenylphenacyl bromide, β-naphthacyl bromide, allyl bromide, methyl iodide and ethyl iodide salts of hexamethylenetetramine indicated that all these compounds, except possibly the last mentioned salt, had an effect of the same order of magnitude as nitrogen mustards in inhibiting the respiration of rabbit bone marrow cells in serum. The effect appeared to be immediate and no induction period was observed. By use of radio phosphorus it was shown that a reduction in the up-take of phosphorus by the cells accompanied the inhibition of respiration. Results of screening tests against sarcomas in mice are to be reported elsewhere.

Other series of compounds in which the similarity to the pyridinium salts is more apparent include derivatives of pyrazine and quinoxaline, which differ from pyridine and quinoline by having a second nitrogen in the ring in place of the carbon and hydrogen in the number 4 position. It might be expected that each molecule of these bases would tend to react with two molecules of organic halide, but it has been found that the products actually obtained have only one quaternized nitrogen. The pyrazinium salts formed readily, although there were wide variations in the time of reaction with different halides, and those having low molecular weight were moderately soluble in water (Bahner and Norton, 1950). The quinoxalinium salts were more difficult to prepare and special precautions were found to be necessary in order to obtain satisfactory yields (Easley and Bahner, 1950). In addition to quinoxaline itself, 6-chloroquinoxaline, 6-methylquinoxaline and 2, 3-dimethylquinoxaline have been used as starting materials. Some forty pyrazinium and quinoxalinium salts have been submitted for

screening tests, but the results are not yet available for publication.

The bases described above all contained only tertiary nitrogen atoms, so that only quaternary salts could be formed. The use of morpholine, piperidine, pyrrolidine, tetrahydroquinoline and tetrahydroisoquinoline which are heterocyclic secondary amines makes possible the preparation and comparison of tertiary amines, and quarternary ammonium salts. Lutz and associates (1947) reported a number of α-phenyl-β-aminoethanols containing such rings, together with some of the corresponding ketones, prepared for testing as antimalarials. Since piperidine, 1,2,3,4-tetrahydroquinoline and 1,2,3,4-tetrahydroisoquinoline may be considered as hydrogenated forms of pyridine, quinoline and isoquinoline, it becomes possible to examine the effect of this simple change upon the activity of several of the compounds which have been reported to damage sarcoma cells *in vivo*. Approximately fifty derivatives of these secondary amines are undergoing screening.

Coming even closer to the pyridine and quinoline and isoquinoline compounds previously reported as active, a number of salts have been prepared by quaternization of halopyridines and haloquinolines, such as 2-chloropyridine, 2-bromopyridine, 3-bromopyridine, 3,5-dibromopyridine, 2-chloroquinoline, 6-chloroquinoline and 4,7-dichloroquinoline. One reason for interest in such compounds is the possibility of tagging them with radioactive halogens. Another is the possibility that halogen atoms in certain positions may increase the biological effects of the substances. As data become available regarding the test results additional compounds are being synthesized to follow up the most interesting leads. For the present it must suffice to state that, as might be expected, the presence of a halogen atom in the 2- or 4- position makes the reaction of the base with organic halides much slower and that the presence and location of the halogen atom does make a substantial difference in the physiological effects.

Several salts of 8-hydroxyquinoline have been prepared in order to learn whether the presence of the hydroxyl group in this position produces any marked change in the activity of the

quinolinium salts. Also several p-fluorophenacyl salts of alkylpyridines have been prepared in order to learn whether the fluorine atom in this position increases the pharmacological effects of the compounds.

While attention has been directed toward the changes in the heterocyclic bases used, some comment should be made concerning other parts of the compounds. In the type formula the portion of the compound marked "B" has been either a $-CH_2-$, group or a $-CH(CH_3)-$ group. The presence of a methyl group in this position retards salt formation. The portion of the compound marked "C" may be H, $-CH_2-$, –CHOH–, –CO–, or C(NOH)–. A large number of variations have been tried in the portion of the compound marked "D" including H, alkyl, haloalkyl, cycloalkyl, phenyl, 4-halophenyl, 3,4-dihalophenyl, 2,5-dihalophenyl, 4-alkoxyphenyl, 4-phenoxylphenyl, 4-phenylphenyl, 4-hydroxphenyl, 3,4-dihydroxyphenyl, β-naphthacyl, 5,6,7,8-tetrahydro-β-naphthacyl, α-naphthacyl and 4-halo-α-napthacyl. There is a tendency for the larger groups to slow down the rate of reaction of the halides and a tendency for certain substituents such as fluorine in the para position to increase the reactivity. The anion may be Cl^-, Br^-, I^-, or $ROSO_3^-$.

Work has been begun on the synthesis of two series of compounds less closely related to the chosen type formula. It will be noted that in this formula there are two rings joined through a chain of two carbon atoms and that one of the rings contains a quaternized nitrogen atom. These characteristics are shared by 2- and 4-styrylquinolinium salts, which also resemble stilbamidines in having two rings joined through an ethylene group. Other workers have prepared such compounds for various purposes, such as use as indicators in acidimetry (Werner, 1920). Some of the earlier preparations are being repeated and additional new compounds are being prepared, through heating an aromatic aldehyde with a quaternary salt of a 2- or 4-methylquinoline or pyridine, according to the method of Phillips (1947).

The condensation of benzaldehyde with quinaldine under the influence of sunlight produces 1-phenyl-2-(2-quinolyl) ethanol (Benroth, 1906). A series of analogous compounds are being

prepared by this method with the expectation of comparing the effects of the quaternized and unquaternized substances.

The work described in this progress report is being continued in the expectation that increasingly useful information will be brought to light as data on the effects of the various compounds in screening tests at the National Cancer Institute, the Sloan-Kettering Institute for Cancer Research, the Midwest Research Institute, the Foundation of Applied Research and other laboratories are correlated and used to point the way in the selection of further compounds for synthesis and study.

Summary

In an effort to obtain additional substances which damage cancer cells selectively, approximately two hundred quaternary salts have been prepared, containing substituted or unsubstituted thiazole, benzothiazole, hexamethylenetetramine, pyrazine, quinoxaline, morpholine, piperidine, pyrrolidine, tetrahydroquinoline, tetrahydroisoquinoline, pyridine, quinoline, and isoquinoline rings. This work is being continued.

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MICROSCOPIC AUTORADIOGRAPHY AS A TOOL IN CANCER RESEARCH

GEORGE A. BOYD*

Radioactive isotopes are, or can be, used in three ways in the attack on cancer: (1) in therapy, (2) in tracing possible chemotherapeutic agents, and (3) in the study of the basic processes of growth. The Geiger-Muller counter and the ionization chamber are the most widely used instruments to measure the radioactivity in cancer research. But another instrument, the photographic emulsion, is now coming into use. This has an advantage over the conventional electronic instruments since it can be used to trace radioactivity to the individual cell or a small collection of cells under the microscope. To date, the cellular studies have been qualitative but quantitative studies are feasible. In this respect it is more suitable for histological tracer work by the pathologist than are the electronically amplified instruments. The following will illustrate the differences in the use of the two types of instruments.

With a Geiger-Muller counter or an ionization chamber, we can measure the dose of radioactivity given to an experimental animal or a patient and the amount excreted. If we remove a part of some organ and process it chemically, we can determine how much has been deposited at the time of sacrifice or the excision of a specimen for biopsy. Thus we measure that which enters and leaves a biological system and that deposited in a bulk of tissue.

This type of information has proved extremely valuable in clinical, physiological, and biochemical research. However, the data so derived pertain to a bulk of mixed cellular components; not to single cells or groups of cells. Thus, there is a lower limit of the area in which radioactivity can be located by these tools. For example, let us consider the measure of the radioactivity in a cubic centimeter of blood. For the sake of calculation, we shall ascribe to this volume an activity of 10^4 disinte-

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grations per ten minutes. This is a fairly active sample when measured with the electronic instruments and would give a sufficiently accurate count in ten minutes.

This ten minute count, while extremely useful for some purposes would not help a histologist studying microscopic localization. If he wished, for example, to study the uptake of C^{14} labeled molecules or P^{32} by normal or abnormal red cells or in one stage of development in the bone marrow, it would be impossible to do so with these instruments as demonstrated by the following analysis. One cubic centimeter of blood would have an order of 10^{10} cells. With an activity of 10^4 disintegrations per ten minutes we would have a mean of one disintegration per million cells per ten minutes. This statistic is the only type of information we can obtain with the Geiger-Muller counter.

A histochemist looking for either the normal or abnormal uptake of radioactivity by a single cell would find it a hopeless task to study individually each of the million cells looking for this one disintegration in ten minutes. You will remember that the one per million per ten minutes is a statistic, and he would know neither when nor in which cell the disintegration would take place. This task is even more hopeless because of the geometrical, mechanical, and electrical nature of the Geiger-Muller tube and counter and the background.

Although blood was used for illustration, the same reasoning applies to tracing an active atom to a glomerulus or tubule in the kidney, a single liver cell or distinguishing between physiologically active and non-active follicles of a thyroid.

Can autoradiography succeed at the cellular level where the Geiger-Muller counter and ionization chamber fail? Yes. It must not be inferred that this is a disparagement of the electronic instruments. It is not. Autoradiography does not supplant these instruments but complements them. The Geiger-Muller counter is used to measure the amount given to an animal and the amount excreted, but the photographic emulsion can be used to locate the activity in specific cells or groups of cells. The autoradiographic technique combines photography and histology through the coupling agent, radioactivity, to

point the way to a systematic study of the microscopic destination of labeled molecules in biological systems.

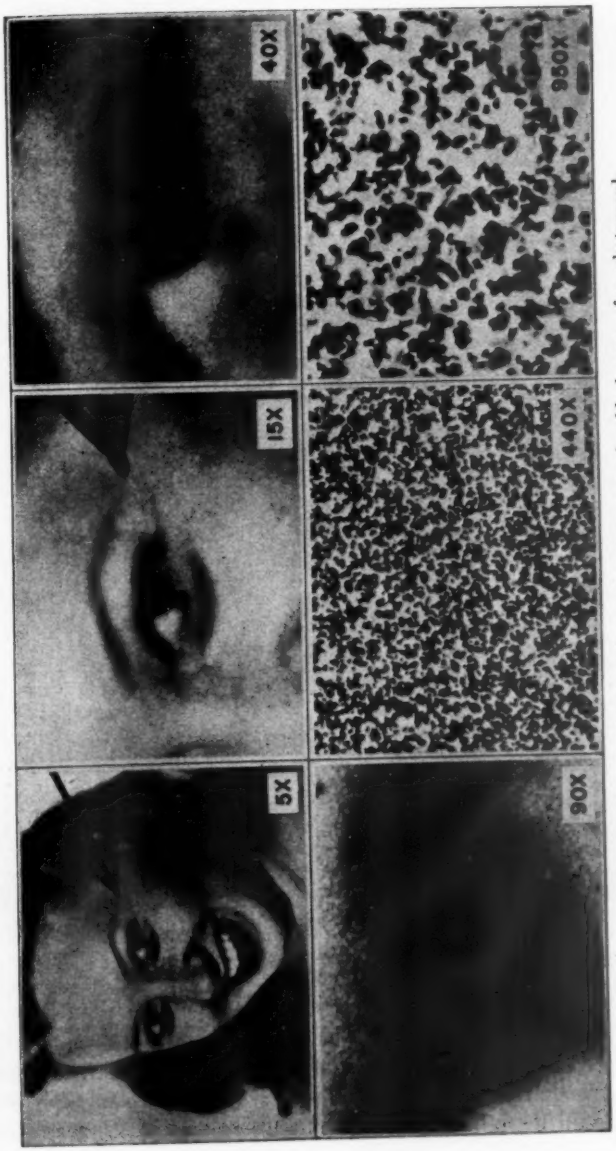
Ionizing rays such as beta rays from a radioactive atom affect a photographic plate in the same manner as X-rays. When a tissue section containing radioactivity is placed in contact with a film or plate radiation from each histological or cytological site containing radioisotopes will produce its own "picture" or autoradiograph. This, then, is another form of histochemistry.

Figure 1 shows in the top left corner a picture of a young lady's head. But let us consider the microscopic construction of the photograph. By concentrating on any black area of the photograph and following it through successive magnifications one may note the solid black area dissolve into individual black grains of silver. Similarly autoradiographs occur of all degrees from solid black at no magnification to a random distribution or pattern of black grains.

In Figure 2 one may observe a grossly black autoradiograph at low magnification. Radiostrontium was given to a young boy having a bone tumor[1]. After amputation of the leg it was sectioned and placed in contact with an X-ray film for some time before development. Comparison of the roentgenogram of the section and the autoradiograph shows the deposition of radiostrontium in an osteogenic sarcoma, the epiphyseal line, and other areas of the bone.

This demonstrates that this element is not selectively taken up by the tumor. From a therapeutic standpoint this is valuable information of a negative type. It indicates that one cannot selectively radiate this bone tumor with strontium without possible damage to healthy tissue. However, the autoradiograph demonstrates a method of study in the search for tumor-specific internal radiators. But at this gross level of detail a Geiger-Muller counter is almost as good as the photographic film. It would have given similar information if the bone had been surveyed in detail.

In contrast to autoradiographs of gross structure where there is less of a choice between instruments the photographic plate is superior to the Geiger-Muller counter in showing the differential uptake of iodine by adjacent, normal, and abnormal



Successive enlargements showing the grain structure of a black area of a photograph.

A B

A. Roentgenogram of section of leg.
B. Autoradiograph of section of leg showing concentration of radiostrontium in tumor and at epiphyseal line.

follicles. This is illustrated in figure 3, showing an autoradiograph of a non-toxic nodular goiter. This photograph was made at a magnification of about 400x. The autoradiograph was made in the following manner: A tracer dose of I^{131} was given

Autoradiograph of a field of non-toxic nodular goiter showing spotty uptake, with deposit chiefly in the smaller follicles. Adjacent small follicles, similar histologically, show no uptake.

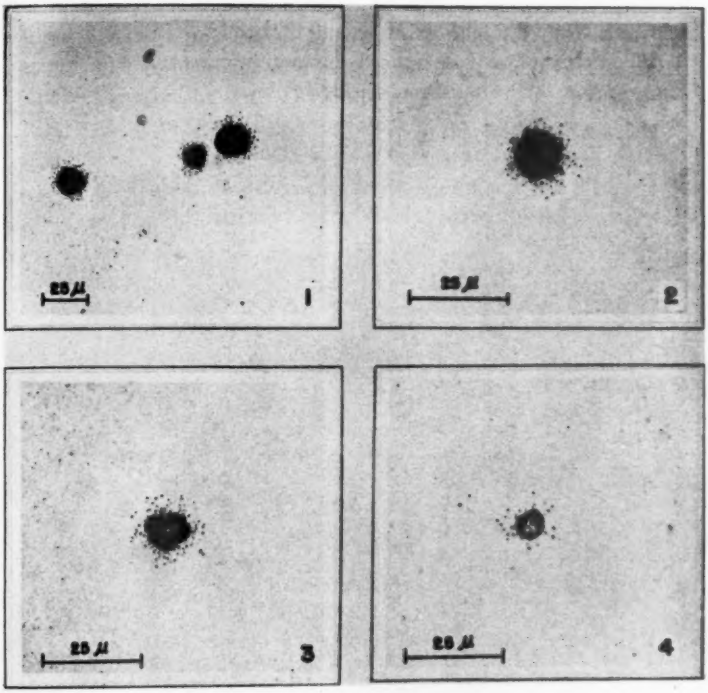
to the patient, a specimen was taken for biopsy later, a paraffin section was permanently mounted on a photographic plate and, after exposure and photographic development, the tissue was stained in situ on the plate.[2] The autoradiograph and the tissue were studied and photographed simultaneously under the microscope.

This autoradiograph is interesting to the pathologist in that it shows a physiopathology where there is no histopathology. It is desirable to trace labeled molecules to single cells in the study of the basic chemical processes in normal and abnormal growth. For example, if one could identify a single blood cell or a bone marrow cell in one particular stage of maturation and at the same time say that it had or had not taken up a labeled amino acid, P^{32}, or some other molecule or atom of interest one could begin to study the production of the formed elements of the blood. This would be of tremendous value in the study of blood dyscrasias. A step toward the realization of this goal has been made.

A group headed by Dr. Kurt Salomon at Rochester was studying hemoglobin synthesis with C^{14} labeled glycine using the ionization chamber[3,4], when the technique of studying the chemistry of a single cell was developed with the assistance of Dr. George Casarett and Miss Agnes Williams. Glycine labeled with C^{14} in the alpha carbon position was injected into a rat. Three microcuries were given and 25 hours later the rat was sacrificed. We attempted to study the bone marrow cells wishing to find at which stage of maturation the glycine was incorporated into the heme. Unfortunately, because of unforeseen technical difficulties, we were unable to do this but we were able to obtain good autoradiographs of individual mature cells. Figure 4 shows these.

In the lower right corner is a photomicrograph of a single red cell and scattered about it are several discrete black specks. These constitute the autoradiograph produced by the C^{14} and presumably the glycine or its metabolic conversion products incorporated into the cell. In the figure of the lower left corner is shown the nucleus of a polymorph surrounded by its autoradiograph. The two upper photographs show the uptake by lymphocytes, photographs at two different magnifications.

By studying the entire smear we found a striking demonstration of one physiological characteristic of the cell types. An entire smear showed only one, two, or three long-lived red cells to contain C^{14}. During the twenty-five hours between injection and sacrifice a small number of red cells pass through the stage



Autoradiographs of C^{14} incorporated in individual blood cells: (1) Field of test blood smear illustrating non-uniform distribution of silver grains and concentration of grains around certain cells (x 440). Cells without autoradiographs are erythrocytes. The exposed test plate from which this photomicrograph was made was soaked in water for 5 min. and developed in a dilute solution of D-19 at 20°C for 25 min. (D—19:H^2O=1:3). (2) Lymphocyte (x 950). (3) Polymorphonuclear leucocyte (x 950). (4) Erythrocyte (x 950).

in which the glycine was incorporated and entered the circulating blood. But a very high per cent of the lymphocytes showed uptake. Thus, the more rapid synthesis of the shorter lived cells could be differentiated from that of the more slowly produced red cells. However, it must be realized that other parameters may have been reflected in the autoradiographs.

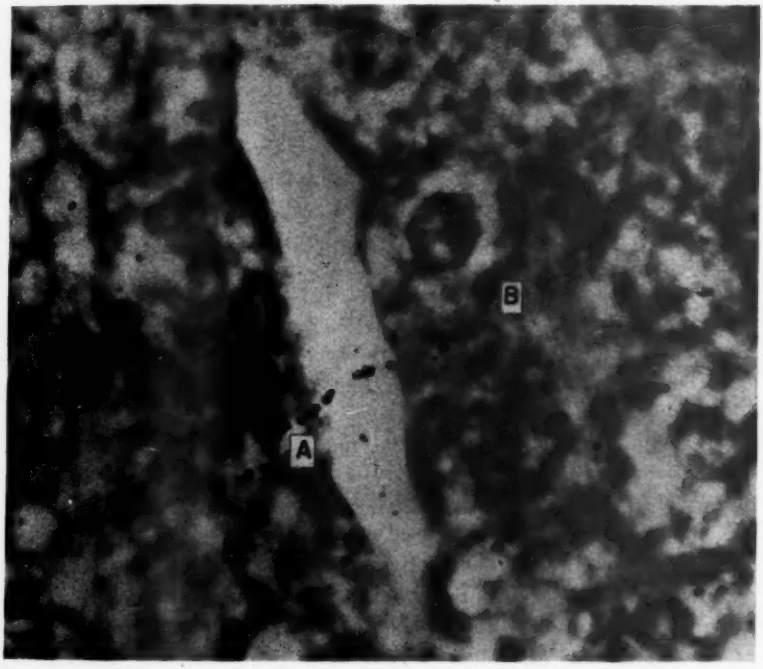
Dr. Morse[5] of Montreal has recently overcome the technical difficulties of bone marrow autoradiographs and succeeded in

making similar autoradiographs of bone marrow cells containing P^{32}. Figure 5 shows his excellent results. This is a basophilic erythroblast from the bone marrow twenty-one hours after 0.5 mc. of P^{32} had been injected.

Autoradiograph of a basophilic erythroblast containing P^{32}. The randomly distributed discrete black specks constitute the autoradiograph. The diffuse black and gray areas constitute the photomicrograph of the stained cell.

Last summer Dr. Levi from Copenhagen helped in the development of a quantitative method[6]. Tissues from the same rat which had been injected with glycine, in the previously mentioned experiment with Dr. Salomon, were used. These were placed on a new emulsion developed by the Eastman Kodak Company for the purpose. Figure 6 indicates the results.

The earlier autoradiographs showed either solid black areas or a group of randomly distributed black specks. Here one



Photomicrograph of an autoradiograph of a liver section from a rat injected with C^{14}-labeled glycine. A composite photomicrograph of a view at tissue level and a mosaic of a beta particle track in the emulsion. The photomicrograph of the tissue was taken at a focal plane just above the tissue-emulsion interface. The composite of the track, starting at the interface and running in three dimensions in the emulsion, was made in the manner presently used for the study of the physics of nuclear tracks. These were superimposed and rephotographed. The increasing grain density along the track shows that the beta particle entered the emulsion near A and stopped (at a lower point in the emulsion) at B. The cell nucleus near the origin of the track has been slightly retouched to show its size relative to the silver grains.

may note a wavy line of black specks. This is an autograph, an autograph of the path of a single beta particle which indicates the C^{14} atom to be located in this cell. At least this is statistically true.

We can never be certain that any one track is caused by a beta particle from the radioactive atom under study. The emulsion also shows, as tracks the degradation electrons of primary cosmic rays. But even with this minor objection, we can obtain detailed quantitative information if we pay due regard to statistics. Thus, to make a quantitative determination it is necessary to count the tracks per microscopic field underneath a section of tissue and subtract from this the concentration of tracks in the emulsion of the same plate but at some distance from the tissue section.

Figure 7 shows the tracks at a lower magnification used in the counting procedure. In this the tissue is out of focus being in a plane above the emulsion containing the tracks. In this particular experiment we found the track concentration to be about nine times the background. This is sufficient for significant tracer work. The determination of the activity of the liver section by this method agreed reasonably well with determinations made with an ionization chamber on larger quantities of tissue.

The molecule we were studying was an amino acid and demonstrates the application of the technique in the study of the fundamental processes of growth. The same technique is applicable to the study of labeled molecules in a search for a compound which would be selectively absorbed by neoplastic tissue. This molecule might be either a chemotherapeutic agent or serve as an internal radiator.

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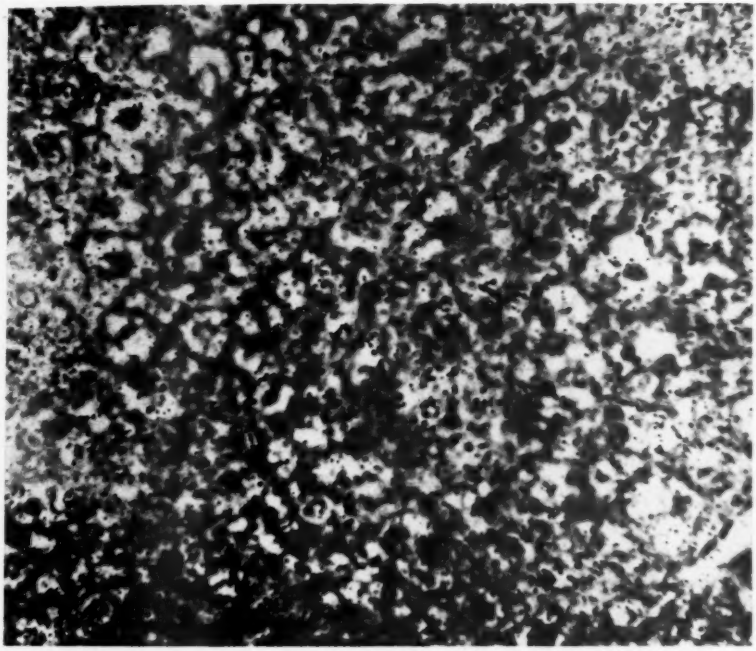

Photomicrograph of an autoradiograph of a liver section from a rat injected with C^{14}-labeled glycine. The short crooked lines of dense black specks constitute the autoradiograph. Each crooked line is a track of a single beta particle. The microscope was focused through the tissue into the emulsion beneath, thus giving poor detail of the tissue but good detail of the tracks.

CANCER THAT IS NOT ALWAYS PLACED IN THE CANCER CATEGORY*

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Despite the fact that the use of the microscope in the diagnosis and classification of cancer has shown a marked increase in cancer in relatively recent years, there are still a number of neoplasms that are cancer microscopically, some of which are capable of metastasizing if given time or have actually metatasized when discovered, that are not always classified as cancer. These neoplasms not infrequently masquerade under terms so ambiguous that even the well-informed physician would not suspect that they belong in the cancer category.

If one is to judge by the written and spoken word of his colleagues, one gains the impression that, not only is relatively little effort being exerted by way of placing certain microscopically proved cancers in the cancer category that not infrequently remain outside of it, but that there is a tendency to take certain cancers out of the cancer category because they show no or only a little tendency to metastasize by the blood or lymph streams, grow slowly, remain at the site of origin for a long time or, in other words, are slow to encroach on the adjacent tissues or break out of bounds.

Cancers that are not always placed in the cancer category may be described in the following terms: (1) pseudomalignant lesion, epithelial restlessness and precancerous lesion; (2) Bowen's disease; (3) adenoma, polyp, papilloma, papillary cystadenoma, papillary adenoma, islet adenoma or insuloma and parathyroid adenoma; (4) benign metastasizing goiter; (5) lateral aberrant thyroid; (6) carcinoid; (7) mixed tumor;

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(8) dysgerminoma; (9) arrhenoblastoma and (10) granulosa-cell tumor.

Pseudomalignant lesions, epithelial restlessness and precancerous lesions may prove to be real cancer that can be correctly termed "intra-epidermal cancer, pre-invasion cancer, convert cancer or carcinoma in situ." I defined carcinoma in situ as follows: a condition in which epithelial cells and their progeny are found in or near positions occupied by their ancestors before the ancestors underwent malignant transformation.

Bowen's disease is typical carcinoma in situ and should always be placed in the cancer category.

An adenoma, especially of the large intestine, may contain a carcinoma usually of a low grade of malignancy. Furthermore, the so-called bronchial adenoma is, as far as my experience goes, without exception a cancer, usually of a low grade of malignancy. A polyp, especially of the large intestine, is usually an adenoma either with or without carcinoma. A papilloma, especially if it is situated in the thyroid gland, breast, ovary, pelvis of kidney, ureter, urinary bladder or urethra, is practically always a cancer. Papillary cystadenoma and papillary adenoma regardless of situation usually turn out to be cancer. Islet adenoma or insuloma of the pancreas is usually a cancer as is parathyroid adenoma and both are usually of the functioning type.

The so-called benign metastasizing goiter is invariably a cancer and not infrequently of the functioning type.

The so-called lateral aberrant thyroid is a metastatic lesion from a cancer of the thyroid gland and usually presents a papillary effect.

Carcinoid, regardless of its situation, is always a cancer.

The mixed tumor, especially of the salivary, lachrymal and mucous glands, regardless of the slowness of its growth, almost without exception contains a cancerous element.

The dysgerminoma, comparable microscopically to the carcinoma of the testis or seminoma, although not as malignant, is always a cancer.

Arrhenoblastoma and granulosa-cell tumor, regardless of the rate of growth, are cancers that frequently function.

In the foregoing presentation, I have called attention to a number of new growths that are cancer or may contain cancer that are not always placed in the cancer category, in an endeavor to return certain cancers to the cancer category and block any attempt, regardless of the reason, to take others out of it.

FACTORS DETERMINING DEPTH DOSE DISTRIBUTION FROM A RUTHENIUM PLAQUE

DOMINIC J. CARA, M.D.

In the treatment of superficial lesions of the body by ionizing radiations it is desirable to have the radiation limited to a particular depth, beyond which normal tissues would be unaffected. If a superficial quality of X-rays is used the normal deeper structures necessarily receive a certain per cent of the incident dose. While this is never as great as the surface dose, unless multiple parts are used, it may be of sufficient quantity to delay recovery, to result in a poor cosmetic result, or to end in necrosis and late radiation changes. However, complete recovery of the deep tissues usually occurs if the radiation has been administered judiciously.

With the advent of radio isotopes it was found that certain of these which were beta emitters could be used as surface applicators, that the radiations were limited to a few millimeters of tissue and that satisfactory results could be obtained in the treatment of certain lesions of the skin, such as small basal cell carcinomas, hemangiomas and warts. To these may possibly be added hyperkeratosis. Low-Beer (4, 5) is the first to use P^{32} in such a manner. P^{32} is a pure beta emitter having a maximum energy of 1.7 MEV with an average energy of .7 MEV. The dose falls to 50% at about .8 mm. of tissue. If malignant diseases of the skin are to be treated by a surface irradiation a better depth dose is a necessity. The half life of P^{32} is only 14 days, a fact which is also an undesirable feature.

Ruthenium^{106} is a product of uranium fission and is the source of a very strong beta radiation with end points at 3.55 and 2.30 MEV. The half life is 290 days. These properties, in addition to others listed below, suggested the possibility that it would be more suitable than any of the others as a surface irradiator.

General Physical and Radioactive Properties of Ru^{106}

Of all the elements found in the fission process Ruthenium^{106} is the source of the highest energy beta particle. Ru^{106} itself

emits only a very weak beta with an energy of about .03 MEV. The immediate daughter element Rhodium[106] with half life of 30 seconds decays to stable Palladium[106] by the emission of beta particles with end points at 3.55 and 2.30 MEV. The former is present in 82% of disintegrations; the latter 18%. Associated with the latter are three gamma radiations: two occur in cascade, each having energies of .51 and .73 MEV respectively. Or, a single gamma of 1.25 MEV occurs in 1 to 2 per cent of the total disintegrations of Rhodium. The decay scheme of Rhodium as determined by Peacock (6) is shown in Figure I.

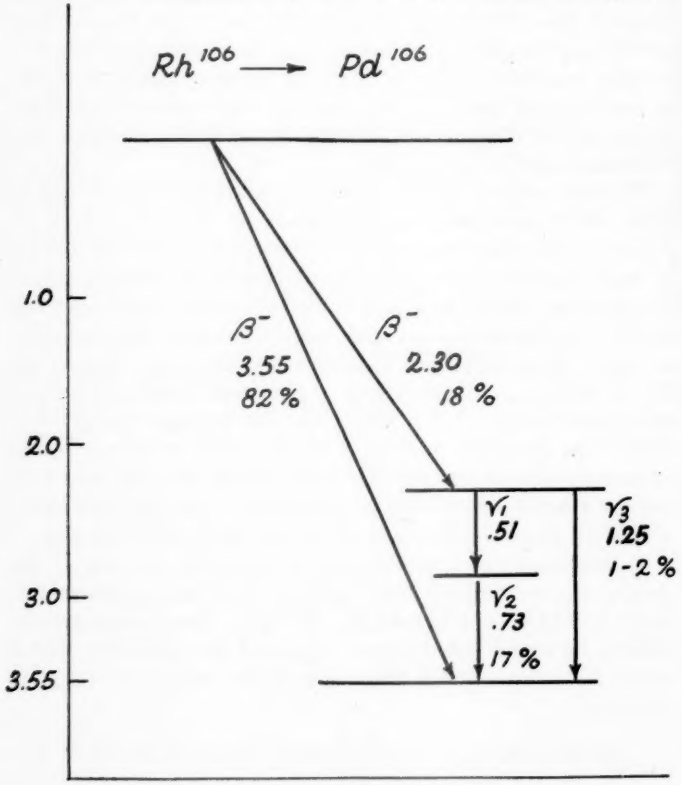

FIGURE 1
Decay scheme of Rhodium (as determined by Peacock).

The element Ruthenium belongs to the Platinum group, and like platinum is a shiny metal. Its ability to be electroplated from solution makes it ideal for the preparation of plaques for surface applicators.[2] Because of its greasy, volatile characteristics it must be protected with a thin plating of some suitable material, not only to prevent loss of the source but also to prevent contamination of instruments and the patient. In the construction of our plaques a thin copper disk was plated on one side with Ru^{106} and a thin plating of gold was used as a protective agent. The gold plating is assumed to be so thin that absorption can be disregarded. The disk is about 1.4 cm. in diameter and .5 mm. thick.

The purpose of our project in the beginning was to determine what exposures were necessary to produce erythema, epilation and necrosis in rabbits and pigs. While such data could not be transferred to human application it would give some indication as to what relative doses would be required to give comparable results in humans. It was further thought that before any biological studies were begun it would be most advantageous to know what the distribution of energy from the plaque was in a tissue equivalent material. Beta ionization extrapaliation chambers were not immediately available and so it was decided that the photographic method would best serve the purpose to determine the distribution of energy in phantom material.

The phantom was made up of thin bakelite strips 4 cm. x 6 cm. x 1.5 mm. thick, between which strips were placed pieces of Eastman noscreen X-ray film .25 mm. thick. A single piece of film was placed on the surface of the phantom which was then covered by black photographic paper to make a light-proof film pack.

A second phantom was made from two plexiglass blocks between which a single film was placed vertically and which was also covered by black photographic paper. This photographic paper was .25 mm. thick, so that at no time do our surface readings represent actual surface dose but the value at .25 mm. within the phantom.

These packs were subsequently exposed to the Ruthenium plaque. After exposure the films were developd in Dektol 72

(Eastman) and the densities of each read from a densitometer. The density values do not represent actual dose delivered in equivalent roentgens. However, if they are converted to percentages of the maximum density then a set of values are obtained which represent the approximate relative dose at any depth. It is an easy matter then to find the conditions of filtration, scatter, distance and collimation necessary to obtain the best depth dose or the least depth dose possible. Such knowledge is of particular importance in treatment of cancer of the skin where one proposes to deliver a predetermined dose to the base of the lesion. The erythema, epilation and skin necrosing doses probably vary for different depth dose distributions and is probably greatest with the most superficial distribution.

Conditions Affecting Depth Dose

An unfiltered radioactive beta source decays with emission of beta particles whose energies range from zero to the maximum. The absorption within a material follows an exponential curve,[3] the slope of which depends upon the absorber and radio element in question.

With unfiltred Ruthenium in contact with the phantom it is found that the greatest energy dose is near the surface within the first millimeter. Table I gives the percentage dose per millimeter depth within the phantom. Figure 2-A shows a set of films so exposed to the plaque. The densities at each level are not homogeneous, but are highest in the center, and the area

TABLE 1

Per Cent of Surface Dose Per Millimeter Depth Within Bakelite Phantom

Depth in Millimeters	A On Surface Unfiltered	B 2 cm from surface Unfiltered	C 10 cm applicator with Filters
.25	100	100.0	100
1.	53	68.0	97.5
2.	29	44.5	90.0
3.	17	28.5	73.0
4.	9	17.5	47.5
5.	5	11.5	29.5
6.			18.0
7.			12.5
8.			8.0

of exposure rapidly decreases with increasing depth. However, to obtain a superficial distribution of energy and yet keep the area at any level homogeneously irradiated it is necessary to raise the plaque at least 2 cm. from the surface. In this case reflection of beta particles is important and a plexiglass block 2 cm. thick with a 2 cm. circular hole serves the purpose of a collimator. The values of the percentage dose are given in Table I and the films are shown in Figure 2B.

It has been shown that a parallel and monoenergetic beam of beta particles, as can be obtained from a betatron, reaches it maximum ionization some distance beneath the surface of the phantom.[7] This is roughly about one-third the total distance of penetration. To take a specific example, a parallel and mono-energetic beam of beta particles of 1 MEV reaches its maximum ionization in water at a depth of about 1.4 mm. The surface dose is about 60% of the maximum value. This dose distribution is, of course, preferable to a simple exponential decline. These conditions cannot be repeated with beta particles emitted from radio elements.[1] In the latter case the slope of the curve is quite steep (Figure 4). However, the depth dose can greatly be improved by using the source at a distance, by interposing thin foils at certain levels to scatter the weaker particles from the beam and by collimating the beam. This cannot be accomplished without considerable loss of intensity.

Simple filtration alone only slightly improves the depth dose because while the stopping power of a filter is greatest for the lower energy particles those of higher energy are also slowed down to a certain degree. For this reason, filtration should be kept at a minimum, being only sufficient in amount as to absorb the very weak beta particles and exerting negligible effect on those of higher energy. Through a process of trial and elimination it was found that two gold foils of 25 mg. per cm.^{2} each placed at 8 and 5 cm. respectively from the surface of the phantom gave the best depth dose curve. This is in conjunction with a third filter of aluminum 50 mg./cm.^{2} placed 2 cm. from the surface. The gold foil absorbs some of the weak particles, but in addition serves to scatter some of the particles of intermediate energy from the beam, resulting in overall improvement of quality. Sufficient air space must exist lateral to the

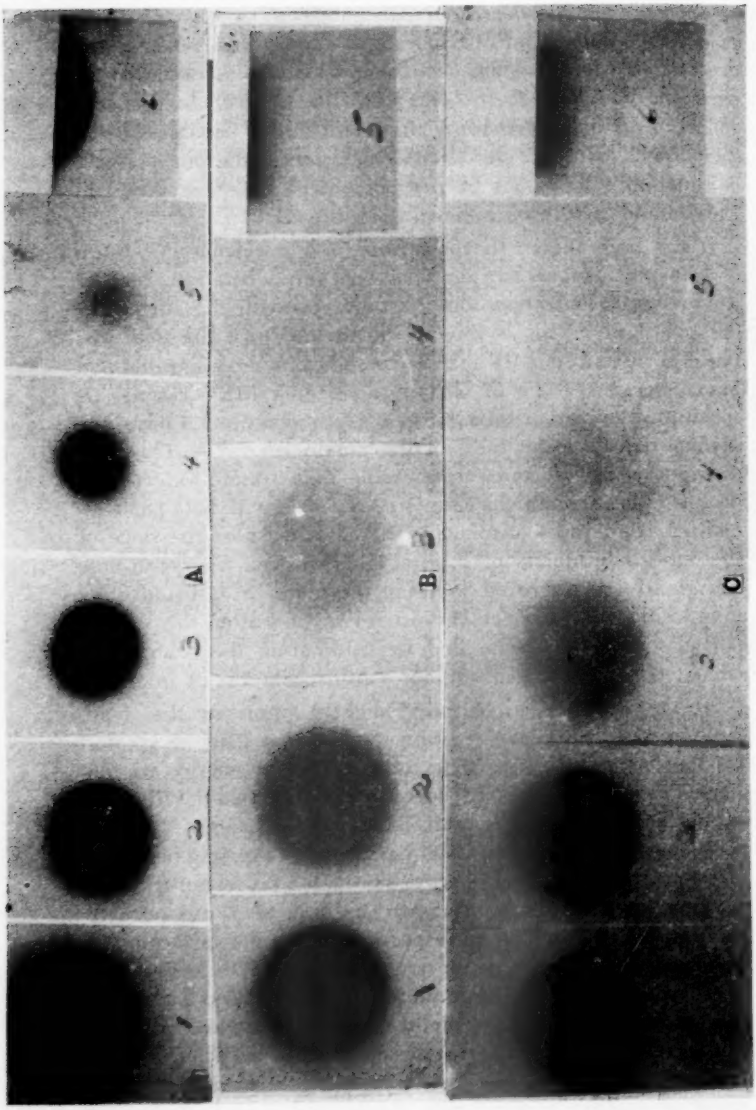

FIGURE 2 (a) (b) (c)
For explanation see discussion.

collimated part of the beam to prevent reflection back into the primary.

A clinical applicator of plexiglass was constructed incorporating the above features. It consists of a hollow cylinder of plexiglass 9.5 cm. in diameter, the plexiglass shell being 15 mm. thick, which is sufficient to absorb all scattered beta radiation. The overall length is 14 cm. However, with the Ruthenium

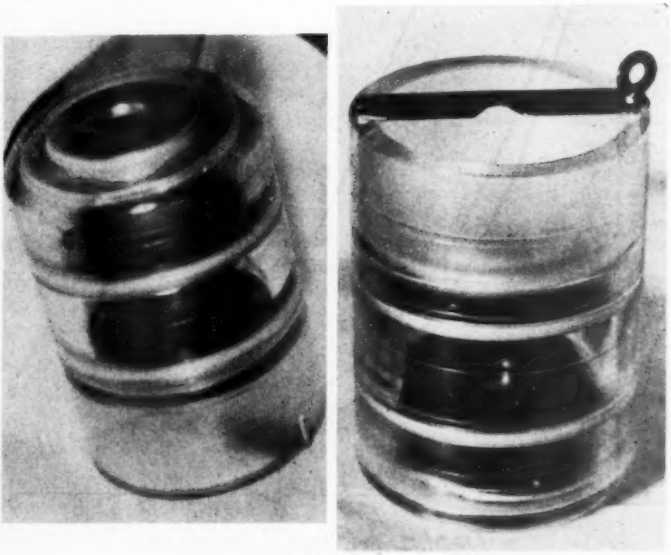

FIGURE 3
Clinical Applicator

in place the distance from the plaque to the surface is 10 cm. The gold foils are held in place at 8 and 5 cm. and the aluminum foil at 2 cm. from the surface by aluminum rings whose centers are 2 cm. in diameter. This gives a fairly well collimated beam of beta particles which is projected on the surface as a circle 2 cm. in diameter. The applicator is shown in Figure 3. The values obtained for per cent dose per millimeter depth are given in Table I and the films in Figure 2C. Unlike those of Figure 2A, the dose at any depth is sharp and homogeneous for almost the entire 2 cm. circle.

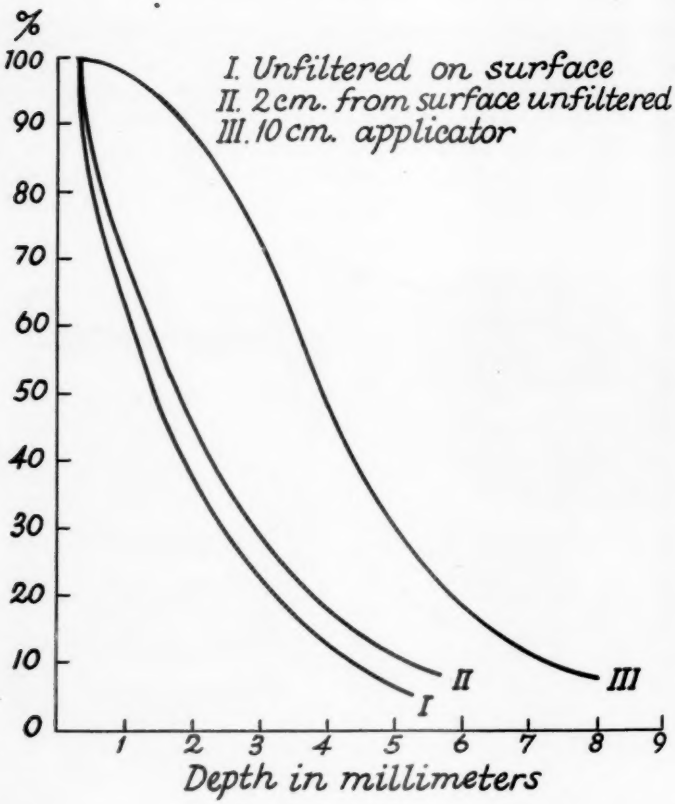

FIGURE 4

Percent depth dose curves in terms of surface dose for Ruthenium under different conditions of filtration, distance and collimation.

The difference in depth dose for the three conditions above described is readily appreciated when reference is made to Figure 4, which shows the respective curves plotted.

SUMMARY

1. Radioactive isotopes which are strong beta emitters offer the possibility of being effective in the treatment of superficial diseases of the skin because of their superficial distribution of radiation.

2. The factors of filtration, distance and collimation have been discussed as they affect the distribution of energy within a phantom of tissue equivalent material from a Ruthenium^{106} source.

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A 1000-CURIE COBALT-60 IRRADIATOR

L. G. GRIMMETT*

It is now more than fifty years ago since radium was first used in treatment of human cancer. During this long period much experience has been accumulated on the possibilities and limitations of this method, and as a result, radium has found a definite and valuable place in the general radiotherapeutic scheme. But radium is costly. With the large-scale production of artificial radioelements in the atomic pile, it is natural that radiotherapists should seek a substitute for radium, something with similar therapeutic properties, but cheaper.

What is required of such a radioelement?

(1) A reasonably long life, preferably measured in years, to eliminate frequent renewal and to avoid troublesome dosage corrections for decay of activity.
(2) Gamma-rays in the range 1 to 2 MeV.
(3) Easily absorbed beta-rays.
(4) Reasonably large cross-section for thermal neutron activation, in order to get a useful degree of intrinsic activity in a short time.

Of the large number of radioelements known today, surprisingly few fulfill all these requirements. Cobalt-60 is one such radioelement. It is produced by simple neutron capture, from natural cobalt which has only one isotope: Cobalt-59:

$$27^{Co59} + o^{n1} \longrightarrow 27^{Co60} + \gamma$$

Cobalt-60 has a half-life of 5.3 years, and decays according to the scheme shown in Figure 1: A beta-particle of energy 0.31 MeV is emitted, followed by two gamma quanta per disintegration, of energies 1.17 and 1.33 MeV respectively. The ionization produced in air by the gamma-rays of Cobalt-60 corresponds to 13.5 roentgens/hour/mc at 1 cm distance. The gamma-rays of Ra (B + C) give 8.3 roentgens/hour/mc at 1 cm, so that a curie of Cobalt-60 is equivalent, in respect of the ionization produced in air by gamma-radiation, to about 1.6 grams

*From the M. D. Anderson Hospital for Cancer Research, Houston. Presented at the Cancer Research Symposium, May 12, 1950.

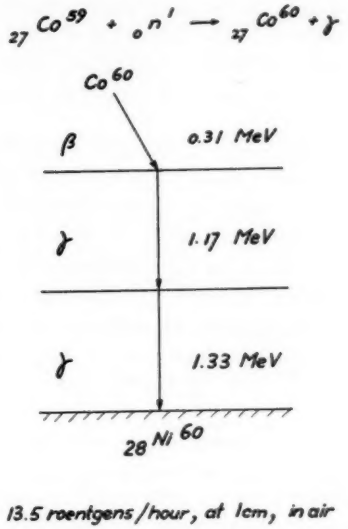

FIG. 1. Decay scheme of Cobalt-60.

of radium. Cobalt-60 can also decay by isomeric transition, but the half life is short (10.7 minutes) and need not be considered further.

In Figure 2, the radiation spectrum of Cobalt-60 is compared with those of Ra (B + C) and a 2 MeV X-ray tube. The two

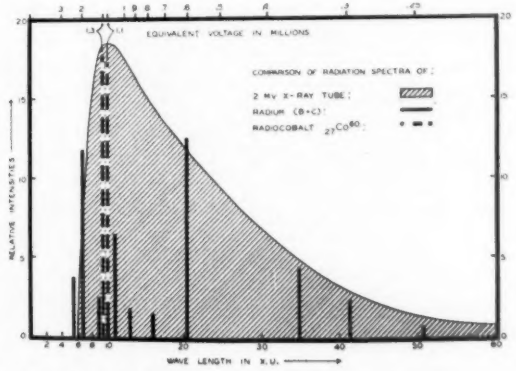

FIG. 2. Radiation spectra of Co-60, Ra (B + C) and 2 Mev X-ray tube.

Cobalt-60 gamma-rays correspond fairly closely with the peak emission of the 2 MeV X-ray tube, and in this restricted sense, Cobalt-60 may be considered "equivalent" to a 2 MeV X-ray tube. The gamma-radiation from Cobalt-60 is essentially monochromatic.

There are three principal ways of using radium:

(1) Radium needles inserted directly into the tissues.

(2) Surface applicators, or plaques, consisting of radium tubes or needles mounted at a short distance from the skin, for treatment of superficial lesions.

(3) Radium beam therapy (teleradium therapy, curietherapy, Fernstrahlenbehandlung), in which a beam of gamma-rays is taken from a larger mass of radium (5–10 grams) at a distance of 10 cms or so from the skin.[1]

Cobalt-60 offers very interesting possibilities in all three techniques, but the present paper deals only with the third.

A typical radium beam unit (Fig. 3), contains 10 grams of radium mounted at a distance of 8.3 cms from the skin. The

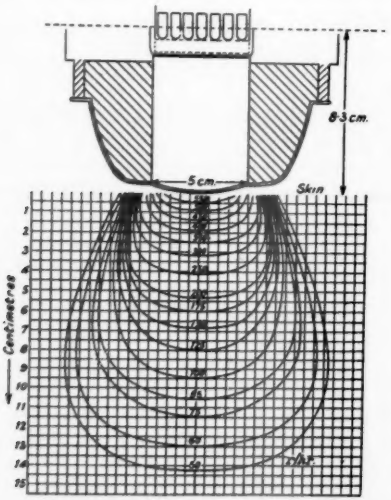

FIG. 3. Isodose curves in water of a 10-gram Radium unit for gamma-ray therapy (Wright, Brit. J. Rad., X, 118, 1937).

beam is canalized by a mass of tungsten alloy. The distribution of ionization in water is shown by the isodose curves.[2] Despite the penetrating power of the gamma-rays, the depth dose at 10 cms depths is only 15%, which is considerably smaller than is obtained with 200 KV X-rays at a distance of 50 cms from the skin. The reason for this curious effect is that the geometrical factors are all-important at small focal-skin distances. To exploit the penetrating power of gamma-rays, one must use radium at a distance of 50 cms or so from the skin (Fig. 4); then the depth dose would rise to over 50%.

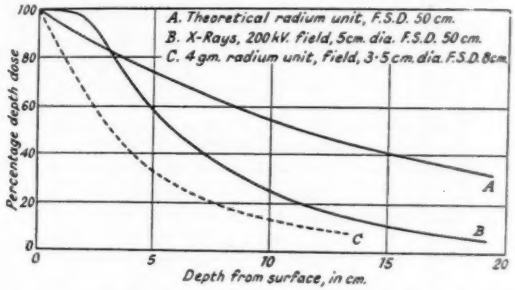

FIG. 4. Comparison of X- and gamma-ray depth doses in water ("Radium Therapy—Its Physical Aspects," C. W. Wilson, Chapman and Hall, London, 1945).

With the quantities of radium available, if the focal-skin distance were increased to 50 cms, the intensity of radiation reaching the body would drop so low as to be biologically useless. To compensate the increase of distance, the quantity of radium would need to be increased to several hundred grams. On account of cost, this is impossible. The use of Cobalt-60 offers a way out; it is quite practical to envisage the use of 1000 curies of Cobalt-60 as a source of radiation. Such a machine would form in effect a *monochromatic* X-ray tube working at about 1.2 MeV.

Before details of design of the Cobalt Irradiator are considered, it might be useful to summarize the reasons why one should strive after high-voltage radiation, rather than be content with 200 KV radiation, with which most of the world's radiotherapy is being done at present.

It is generally accepted nowadays that radiation corresponding to energy greater than 1 MeV gives better clinical results in treatment of malignant disease than 200 KV radiation. It is not difficult to find physical reasons why this should be so: (Fig. 5) shows how ionization builds up below the surface of

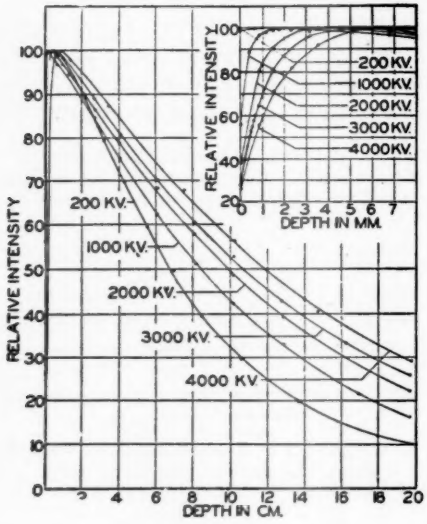

FIG. 5. Depth dose curves for X-rays, 200 KV to 4 Mev (Trump and Cloud, Am. J. Roent., 49, 4, 1943).

the body when different voltages of radiation are used. With 200 KV radiation, the maximum ionization lies very close to the surface. As the voltage of the radiation increases, the position of the ionization maximum moves down into the body, away from the surface, and so lessens the chance of damage to the growing skin cells. The uppermost layers of the skin have a high sulphur content; at the lower voltages there can be appreciable photoelectric absorption, which at voltages above 1 MeV is quite negligible. Depth dose is greater at the higher energy radiations on account of greater penetration, and total body absorption is less at higher energies, partly because of the

increased penetration, and partly because of the smaller tendency to scatter out of the geometrical beam. Hence the constitutional disturbances will be less at higher energies.

Another important point is that the relative energy absorption in bone, muscle, and fat is a function of energy of radiation (Fig. 6). At low energies, absorption in bone measured in

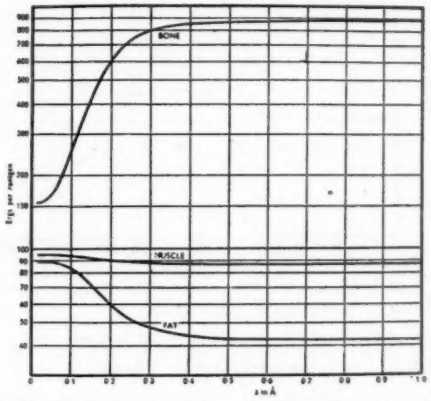

FIG. 6. Comparison of true energy absorption in bone, muscle, and fat at various wavelengths (Spiers, Brit. J. Rad., XIX, 218, February, 1946).

ergs per roentgen delivered, is very much higher than in the soft tissues; as the energy of the incident radiation increases, bone absorption diminishes, and tends to approach the absorption in the soft tissues. In other words, the use of high voltage diminishes the possibility of bone necrosis. All of these factors just outlined are of a purely physical nature. There may be also biophysical factors to be considered. For example, it is possible that there may be some biological superiority inherent in the shorter wavelengths. Now the distinguishing feature of "quality" of radiation from the biological standpoint is the linear density of the ionization produced along the tracks of the ionizing particles.

The ion density per micron of tissues produced by different kinds of radiation is shown in Figure 7. There is a rapid change of linear ion density in passing from 200 KV to 1 MeV radiation. If there is anything in the notion of the superiority of the

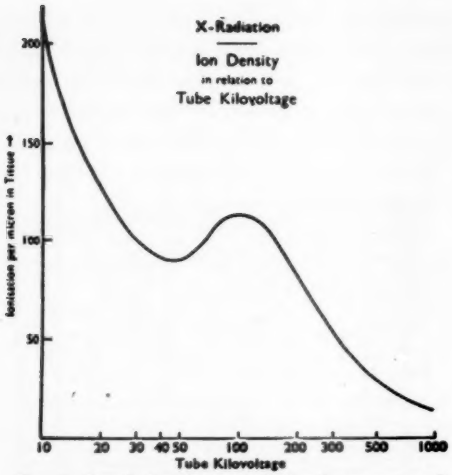

FIG. 7. Graph showing correlation between speed of particle and linear ion density.

higher energies, then it is apparent that practically all the benefit possible will have been attained at 1 MeV.

If then, for all these reasons, physical and biophysical, it can be granted that there are advantages in the use of high energy radiation, then Cobalt-60 with its monochromatic emissions at 1.1 and 1.3 MeV might be expected to show better clinical results than either radium or 2 MeV X-rays, because its radiation spectrum is wholly free from the soft components which exist in the radium and X-ray spectra.

With those ideas in mind, the Oak Ridge Institute of Nuclear Studies, and the M. D. Anderson Hospital for Cancer Research, decided to co-operate in building a 1000-Curie Cobalt-60 Irradiator for experimental radiobiology and ultimately for treatment of human cancer, and as a result, some general basic principles in design soon emerged.

The principal requirements are:

(1) Simplicity and safety in loading the Cobalt-60 into the machine.

(2) Adequate protection for operators (an "off" position of the gamma-ray beam), so that it will be possible to adjust patients for treatment without exposure.

(3) A simple arrangement for turning gamma-ray beam "on" and "off."
(4) The machine must be flexible; it must be possible to raise and lower, and orient it in any desired direction.
(5) Provision for fitting the usual clinical accessories, such as direction indicators, range finders, etc.

The pneumatic system of propelling the radioactive material by air pressure to and from a storage safe was considered and rejected because it may on rare occasions break down. A breakdown with 1000 curies of Cobalt-60 would be intolerable.

Tentative preliminary designs were drawn up in October 1949. Subsequently in February 1950, the Atomic Energy Commission convened a meeting in Washington for discussion of the general problems associated with the use of a large Cobalt-60 Irradiator. The meeting was attended by representatives of hospitals and industry.

Many designs were put forward (Figs. 8 and 9), but we still

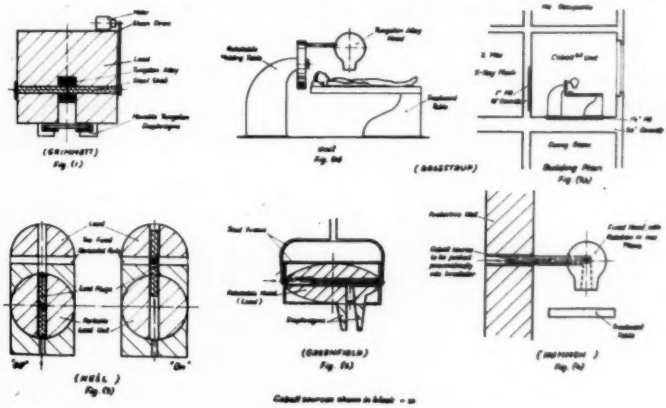

FIG. 8. Cobalt-60 Irradiator designs.

preferred our own as being mechanically simplest. It is shown schematically in the upper left hand corner of Figure 8; the cobalt is mounted on the rim of a metal disc which can be rotated by remote control inside a large mass of protective metal so as to screen the cobalt completely, or expose it through a channel below. The dimensions of the containers required, in various

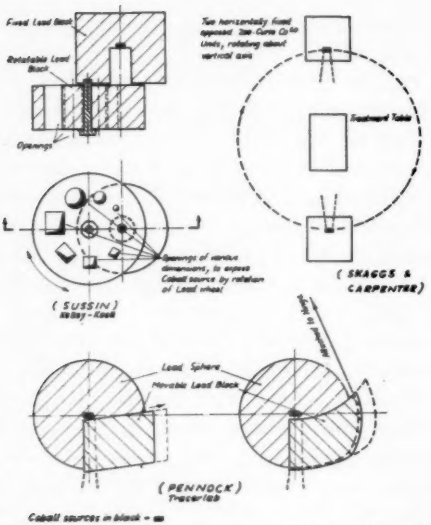

FIG. 9. Tentative designs for a large Cobalt-60 Irradiator.

metals, in order that the radiation at the surface of the container shall not exceed the permissible dose rate of 0.3 roentgens per week, are shown in Figure 10. Uranium would give the

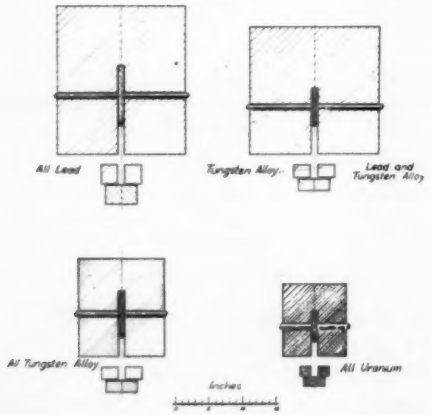

FIG. 10. Comparison of sizes of containers for 1000 curies of Co-60 in different metals to reduce the dose rate to 6.25 milliroentgens/hour at the surface.

most compact, and also the lightest machine, but on account of the difficulty of securing a large enough supply, it was decided that tungsten alloy, an alloy of 90% W, 5% Cu, and 5% Ni, developed by the General Electric Company of England, would be the best choice. It has a density of 16.5 gm/cm^3, is tenacious, and readily machineable (3, 4). There are certain practical difficulties to be overcome before the tungsten alloy can be obtained in large pieces, put we are hopeful of success.

An important point which must be taken into consideration is the penumbra of the beam.

Figure 11 shows the penumbrae obtained from cobalt sources

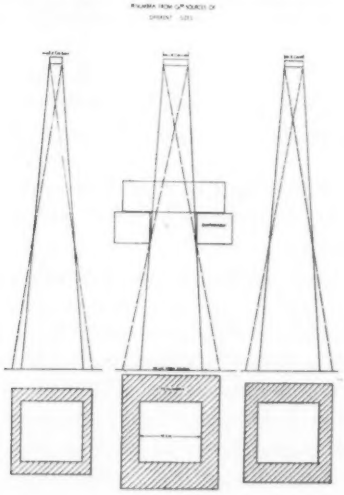

FIG. 11. Penumbra for various sizes of Co-60 source.

of different sizes, when a movable diaphragm system 15 cms above the skin is used. It is clearly advantageous to use a Cobalt-60 source of the smallest dimensions possible, preferably not more than 2 cms cube. To get the desired activity into this small volume, it will be necessary to use a high neutron flux, such as is available in the Canadian pile at Chalk River.

Figure 12 is a schematic drawing of the final design. Interchangeable diaphragms will be inserted into the lower end of the unit to vary the area of the treatment field. A lamp and

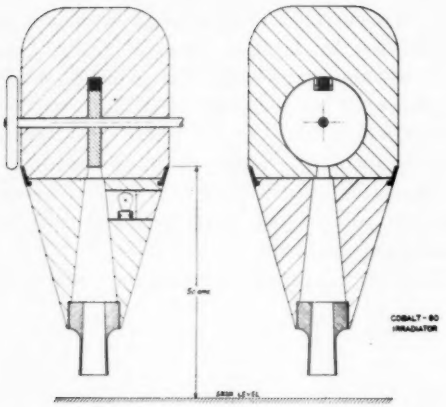

FIG. 12. Schematic drawing of 1000-Curie Co-60 Irradiator.

mirror will be so arranged as to delimit the radiation falling on the skin. The dose rate on the skin will be approximately 90 roentgens/minute.

It is our eventual hope to produce a simple, cheap, and reliable machine, needing no servicing or replacements, apart from the replenishing of the source every five years or so, which will enable monochromatic gamma-rays to be tried for the first time in cancer treatment. The cost is difficult to estimate at this stage, but will probably be in the region of $30,000.00. It would seem to be a sound way of using atomic products, which should bring the benefits of high-voltage radiation within the reach of the ordinary hospital.

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PRELIMINARY DISTRIBUTION STUDIES OF $_{31}Ga^{72}$ IN THE WISTAR RAT*

E. R. KING,† AND JESSE D. PERKINSON

The distribution of subcutaneously injected radiogallium in rats, rabbits and dogs has been reported by Dudley (1, 2, 3, and 4). He has also reported on the toxicity of gallium salts in the form of gallium lactate and gallium citrate, using intravenous and subcutaneous routes of administration (5, 6). In these studies Dudley has reported a high uptake of gallium in rapidly growing bone such as the teeth and jaws of rats, and the ends of the long bones of growing rats, rabbits and dogs. He has also reported an early excretion via the urine. He states that subcutaneously injected gallium is relatively non-toxic in laboratory animals, but the intravenously injected gallium in the form of gallium citrate, is fairly toxic for larger animals, such as the dog and the goat. The toxic effects in the latter case are thought to be due to the citrate and not to gallium toxicity.

The purpose of this paper is to present a preliminary report of a study of the metabolism, distribution and excretion of gallium^{72} in the Wistar rat.

Physical Characteristics of Gallium—Stable gallium metal is a mixture of 60% gallium^{69} and 40% gallium^{71}. Upon pile irradiation these isotopes both undergo an (η, γ), reaction, yielding Ga^{70} and Ga^{72}, in about the same respective amounts. Both of these latter isotopes are radioactive, and both are beta and gamma emitters. The Ga^{70} has a twenty minute half life, decaying to stable germanium^{70}. The Ga^{72} has a 14.3 hour half life and decays to stable germanium^{72}. The gallium is used several hours after removal from the reactor and hence

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*The gallium salts used in this problem were furnished by Comdr. H. F. Dudley, MSC, USN, Navy Medical Research Institute, Bethesda, Maryland. The opinions or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or the endorsement of the Navy Department. Presented at the Cancer Research Symposium, M. D. Anderson Hospital for Cancer Research, Houston, May 12, 1950.

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practically all of the short half life gallium has decayed. The formulae for these reactions are:

$$Ga^{69}\ (\eta,\gamma)\ Ga^{70};\ Ga^{70} \xrightarrow[\gamma]{\beta-} Ge^{70}$$

$$Ga^{71}\ (\eta,\gamma)\ Ga^{72};\ Ga^{72} \xrightarrow[\gamma]{\beta-} Ge^{72}$$

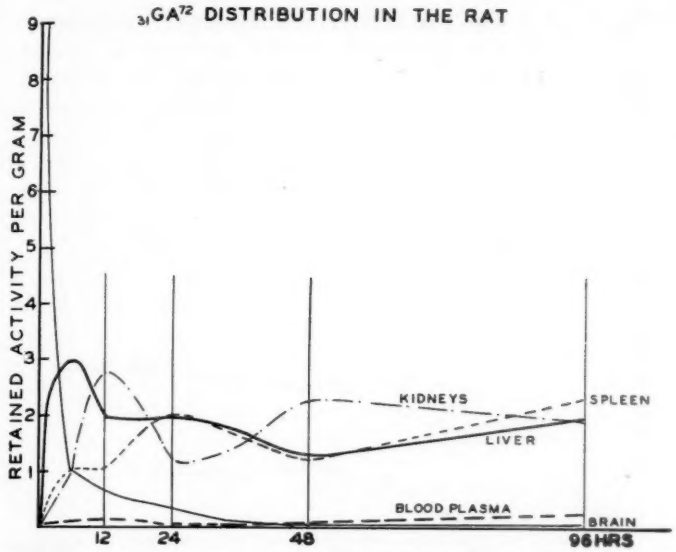
Ga^{72} has a very complex decay scheme which is not completely known. Haynes (7) has published a partial decay scheme, and lists four principal beta spectra and nine principal gamma emissions. Approximately 40% of the beta particles have an energy of about 2.5 mev, and 70% of the gamma rays have energies of over 1.5 mev. The final energy loss of all emissions results in a .84 mev gamma ray with a 100% intensity.

Technique

The radiogallium was irradiated in the Oak Ridge reactor and was contained in carrier gallium with a specific activity of about .4 mc./mgm. gallium metal at the time of injection. Rats used were growing males weighing between 250 to 300 grams. The isotope was injected as an acid solution of gallium chloride into the tail vein of the animal. The dose was adjusted so that about 500 counts/minute/gram of animal were present in the animal at time of sacrifice. The animals were sacrificed by vivi-perfusing them at 6, 12, 24, 48 and 96 hours after injection. Thirty-five samples of different tissues and organs were obtained and were wet ashed with concentrated nitric acid. Urine and feces were collected daily. Radio-assays were made, doing beta counting with a horizontally placed thin-walled dipping tube, using wet samples of 10 ml. each in a small petri dish. All radiation determinations made were corrected for decay back to "0" time which was taken to be the time of injection. A simple table of exponentials for radioactive decay was used for these calculations (8).

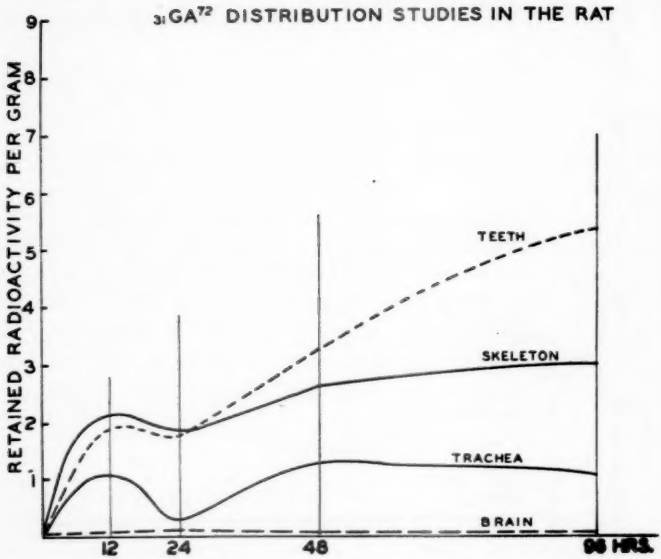
Results

The uptake of gallium in non-osseous tissue was greatest in the kidney, liver, and spleen (Figure 1). The blood plasma



showed a rapid fall of gallium. The erythrocytes did not appear to pick up any of the element. The liver and spleen were demonstrated to have an early uptake of gallium within the first day, than a decrease in the gallium content but with a later rise. The kidney also showed the early uptake, then decrease, but also presented another rise at 48 hours, and a gradual fall after that period.

The teeth, skeleton, and trachea also present the early rise in gallium level at about 12 hours, then a decrease in the level, with a more gradual rise up to 96 hours (Figure 2). The teeth, which in the young rat are probably the site of the most intense osteogenic activity, had the highest activity of radiogallium. It is possible that the rather high level of activity in the trachea may be accounted for by the cartilage present. The skeleton demonstrated a deposition of gallium, at the end of 96 hours, of about 3% of the retained activity per gram of bone. The deposition of gallium in the brain has been added to Figures 1 and 2 to demonstrate the body-background level of the radioisotope. The values given represent the per cent



of the retained activity (total activity minus the activity excreted) per gram of tissue.

Table 1 gives the values for the per cent of the retained dose for certain total organs and tissues measured. It is noted

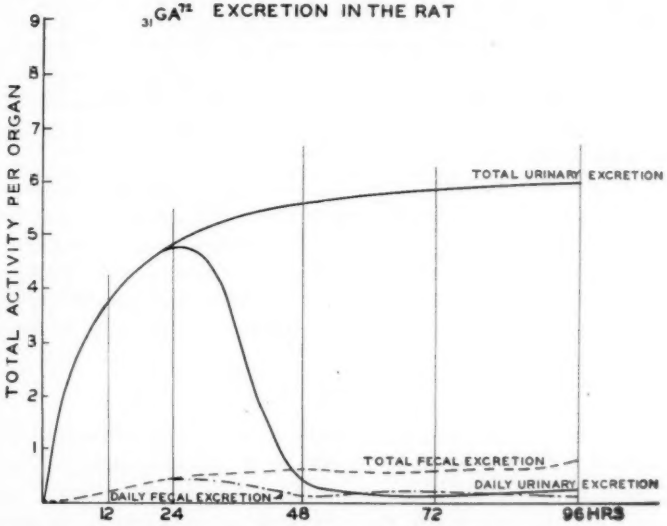
TABLE 1

$_{31}$Gallium^{72} Distribution in the Rat

Time sacrificed	6	12	24	48	96
No. animals used	5	5	4	5	5
ORGAN	% Retained Dose Organ	% Retained Dose Organ	% Retained Dose Organ	% Retained Dose Organ	% Retained Dose Organ
Blood Plasma	8.92	6.32	3.20	1.17	0.75
Teeth	0.24	0.27	0.27	0.69	1.04
Bone	29.65	37.78	31.18	49.30	53.98
Trachea	0.07	0.09	0.04	0.11	0.11
Spleen	0.56	0.93	1.10	0.88	1.50
Liver	27.82	21.61	22.92	15.66	20.47
Kidney	1.37	6.08	2.50	6.34	6.32
Brain	0.03	0.03	0.02	0.03	0.03

that the entire skeleton has a total uptake of over 50% of the retained gallium at the end of 96 hours, the liver has about 20% of the retained activity. The brain is again included to illustrate the body-background level. The remainder of the organs and tissues assayed were not of high enough activity to be significant in a distribution study.

The primary means of excretion of gallium is via the kidney, with about 60% of the total dose excreted within four days (Figure 3). However, about 50% of the given dose is



excreted the first day (Table 2) and the daily excretion rate of the kidney after the first day is quite low. The excretion via the feces is about one twentieth that of the kidneys, with slightly over 4% of the dose appearing in the feces the first day, and with smaller amounts on succeeding days.

The possibility of the injected gallium chloride acting as a protein precipitant and forming a colloidal material should be mentioned. If true it would explain the high levels of deposition of gallium that was found in the reticulo-endothelial organs. However, the pattern of the distribution of the intravenously injected gallium is quite similar to the distribution

TABLE 2

$_{31}Gallium^{72}$ Excretion Studies in the Rat

Time of Collection (Hours)	24	48	72	96
No. Rats Studied	14	10	5	5
		Percent of Total Dose Excreted In:		
Total Urine Output	47.96	55.90	57.35	59.90
Per Urine Sample	47.96	2.95	1.28	2.55
Total Fecal Output	4.22	6.33	6.49	7.57
Per Fecal Sample	4.22	1.71	1.84	1.48

of subcutaneously injected gallium citrate as reported by Dudley. Further studies of gallium distribution in other animals using various gallium salts are now being undertaken by our group.

These facts of early deposition in tissues of high osteogenic activity and early elimination in the urine, plus the desirable physical properties of Ga^{72} (i.e. short half life, energetic beta emissions, and availability through pile irradiation) warrant continued studies on experimental animals, and man in regard to possible uses as a diagnostic tool or in therapy of bone malignancies.

SUMMARY

1. On a gram basis the greatest deposition of gallium at the end of four days is in the teeth, with the next highest levels found in the bone, kidney, spleen, and liver respectively.

2. At the end of four days the greatest deposition of gallium per total organ is in the skeleton and next the liver.

3. About 60% of the total injected dose of the element is excreted in the urine, with about 50% of the dose excreted via the kidneys the first day. The urine excretion is 20 times the fecal excretion.

4. The gallium when injected as an acid solution of gallium chloride appears to be deposited primarily in areas of osteogenic activity as teeth and growing bone, and in organs of the reticulo-endothelial system. The possibility of gallium acting as a protein precipitant and the colloid thus formed being picked up by cells of the reticulo-endothelial system has been mentioned.

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CO-OPERATIVE EFFECTS OF ENDOCRINOLOGICAL FACTORS AND PROCESSES OF AGEING IN PRODUCING ADENOMA-LIKE STRUCTURES IN RATS

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Five experiments were performed on 201 female and 180 male rats, the age of which in different experiments varied from 17 to 27 months. Half of the male rats were castrated about a month before the beginning of the experiment. Tables 1 to 3 show, for each group of rats, the percentage of animals with hyperplastic structures, and the combinations of hormones used. In these tables simple meta-hyperplasias, adenoma-like structures and true adenomas were counted together for each group, and termed as meta-hyperplastic structures. The number of animals in each group is small, and therefore the percentage figures in the tables indicate only a comparative frequency of the structures in various groups of present experiments. The following hormones, singly or in combinations, were administered to the rats in the treated groups: androgens (androsterone, testosterone propionate), oestradiol benzoate-butyrate, progesterone, desoxycorticosterone acetate, thyroid hormone, and, in the form of a concentrated extract, anterior hypophysis hormones.

From the age of about 20 months in some of the untreated rats of the breed used, a spontaneous development of meta-hyperplastic structures of various sizes was found in the thyroid, adrenals, pancreas, thymus and liver. In no case, however, did they become adenomas.

The harmones used increased in various degrees the incidence or development, or both the incidence and development, of meta-hyperplastic processes in the treated rats up to the stages of comparatively large adenomo-like structures or true adenomas, not observed in the untreated animals. It is necessary to emphasize that in senescent rats the neoplastic structures were produced not only by oestrogens, but also by other

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TABLE 1

Experiment 1. Percentage Occurrence of Meta-Hyperplastic Structures in Organs of Rats Treated with Anterior Hypophysis Extract and Other Hormones. Rats about 17 months old.

Groups Rats aged about 17 months	Number of rats in groups	Percentage of structures in Thyroid	Adrenals	Pancreas	Thymus	Liver
1. Controls	8	0	0	0	0	0
2. Progesterone + Thyroid hormone	7	0	0	14.3	28.6	14.3
3. A.H. extract 3 mg.	7	0	0	14.3	0	14.3
4. A.H. extract + thyroid hormone	7	0	0	14.3	14.3	0
5. A.H. extract + thyroid hormone + progesterone	7	0	0	0	28.6	42.9
6. A.H. extract 3 mg. + doca	7	14.3	0	14.3	0	0
7. A.H. extract + androsterone	8	0	12.5	12.5	0	0
8. A.H. extract + androsterone + doca	9	33.3	0	33.3	11.1	22.2
9. Androsterone + doca	7	28.6	0	0	28.6	0
10. A.H. extract 6 mg.	6	0	0	0	0	33.3
11. A.H. extract 6 mg. + thyroid hormone + progesterone	7	0	0	14.3	28.6	0

TABLE 2

Experiments 2 and 3 Pooled. Percentage Occurrence of Met-Hyperplastic Structures in Female Rats Treated with Sex and Thyroid Hormones. Rats about 22–27 months old.

Groups Rats aged 24–27 months	Number of rats in groups	Percentage of structures in Thyroids	Para-thyroids	Adrenals	Pancreas	Thymus	Liver
1. Controls	17	5.9	0	0	5.9	35.3	17.6
2. Androsterone	6	16.7	0	0	16.7	0	33.3
3. Testosterone prop.	16	12.5	0	6.3	25.0	43.8	25.0
4. Thyroid hormone	12	0	0	0	25.0	66.7	41.7
5. Oestradiol B.B.	11	36.4	0	0	9.1	45.5	27.3
6. Oestradiol B.B. + thyroid horm.	6	0	0	0	0	66.7	50.0
7. Oestradiol B.B. + progesterone	15	6.7	0	0	0	40.0	26.7
8. Oestradiol B.B. + progest. + thyroid hormone	6	0	16.7	0	16.7	50.0	50.0
9. Oestradiol B.B. + progest. + thyroid horm. + androsterone	16	6.3	0	0	12.5	62.5	25.0
10. Oestradiol B.B. + progest. + thyroid horm. + testost. prop.	16	12.5	0	0	25.0	62.5	37.5

cell growth stimulating hormones used in the present experiments (Tables 1–3). Adenomas were found in some of the treated rats in the thyroid, parathyroid, adrenals and thymus, while comparatively large adenoma-like structures were observed in the pancreas and liver. The photomicrographs 1–10 illustrate some of the results obtained.

The various forms of the structures observed in different rats showed a gradual transition, through many intermediate stages, of simple meta-hyperplastic nests of few cells into large adenomas with a disorganized growth and form of the cells. Therefore, the results obtained confirm the fact, observed also in human beings, that the smallest meta-hyperplastic nests of cells should be considered as the latent quiescent centers of neoplasms. From this point of view, a detailed investigation of the causes producing these minute epithelial meta-hyperplastic nests and the mechanism of their development in various organs appears to be of great importance.

The results obtained also indicate that there is a co-operative activity of ageing processes and hormonal overstimulation in producing or enhancing meta-hyperplastic structures and adenomas, since they were found in senescent rats but not in the young or adult animals. Therefore, in some special conditions (in senescence, in present experiments), stimulating hormones might become causative factors in the development of adenomas.

The number of rats in the different groups was considered not sufficient to make a comparative assay of the adenomagenic action of each of the hormones, or their combinations used in the present experiments. Most probably, as in the case of mice different strains of rats will prove to be susceptible or resistent to the development of the various tumors. In some of the rats there was simultaneous development of small meta-hyperplasias in certain organs with adenoma-like structures in other organs, or of these two structures with adenomas. It was, however, impossible to ascertain which hormones or their combination were especially active in producing these growths simultaneonsly in various organs.

On the basis of the experiments performed, it is possible to suggest the following explanations of tumor development.

TABLE 3

Experiments 4 and 5 Pooled. Percentage Occurrence of Meta-Hyperplastic Structures in Male Rats Treated with Sex and Thyroid Hormones and Desoxycorticosterone Acetate (Doca). Rats about 20–21 months old.

Groups Rats aged 20–21 months	Number of rats in groups		Percentage of structures in									
			Thyroid		Adrenals		Pancreas		Thymus		Liver	
	Norm.	Castr.	Norm.	Castr.	Norm.	Castr.	Norm.	Castr.	Norm.	Castr.	Norm.	Castr.
1. Controls	13	13	23.1	15.4	17.7	0	0	7.7	30.7	30.7	53.8	30.7
2. Testost. prop.	12	12	16.7	8.3	25.0	33.3	0	41.7	25.0	33.3	58.3	33.3
3. Thyroid hormone	12	11	16.7	18.2	25.0	27.3	8.3	9.1	0	18.2	50.0	36.4
4. Oestradiol B.B.	9	7	22.2	28.6	33.3	42.9	33.3	0	44.4	28.6	33.3	42.9
5. Thyr. horm. + oestradiol B.B.	6	6	0	50.0	50.0	33.3	0	16.7	16.7	66.7	33.3	83.3
6. Thyr. horm. + oestradiol + testost. prop.	13	11	23.1	18.2	15.4	0	15.4	18.2	38.5	27.3	53.8	27.3
7. Doca	6	7	0	28.6	33.3	14.3	0	0	0	28.6	33.3	57.2
8. Doca + thyroid hormone	7	6	0	0	14.3	0	14.3	0	14.3	33.3	57.2	50.0
9. Doca + testost. prop.	6	7	16.7	0	16.7	0	0	14.3	0	0	0	71.4
10. Doca + testost. prop. + thyroid hormone	7	5	0	60.0	0	0	0	20.0	14.3	0	57.2	80.0
11. Four hormones	6	7	33.3	42.9	0	0	0	42.9	33.3	0	50.0	57.2

In the living organism, the cells which build the organs and tissues of our body grow in their number, form, structure and mutual disposition with the most remarkable constancy, in every smallest detail true to type. So far we do not know the mechanism which controls this truly miraculous development and growth of cells.

With ageing, the organs decrease in their relative size and weight, and all the functions of the organism decrease in efficiency. The function controlling the normal cell growth should also weaken. Consequently, and because of this, in the organs of old animals, minute nests of few cells appear which in their structure, disposition and growth differ considerably from normal cells (meta-hyperplasias).

These metaplastic nests were not observed in young or adult rats, but in senescent or senile rats only. These nests usually remain quiescent and therefore harmless. When senescent rats are treated with the cell growth stimulating hormones, a reversal of some atrophic ageing organs towards, or to, the level observed at a younger age occurred. Simultaneously, however, with these hyperplastic and hypertrophying, apparently favourable antiageing effects on the normal cells of the organs, the nests of metaplastic cells are also stimulated (by the hormones used) in their growth. Since they, however, were abnormal, they developed into tumours. Some of these tumours had a structure similar to that of carcinomas.

From the point of view of this hypothesis, it is possible to explain the development of tumours even in young individuals by an exceptional occurrence of these minute metaplastic nests, due to some defects of the cellular growth controlling mechanism.

Most probably any other cellular growth-stimulating factor may produce similar adenomagenic effects. In such a case, various non-specific factors might prove to be tumourogenic causes.

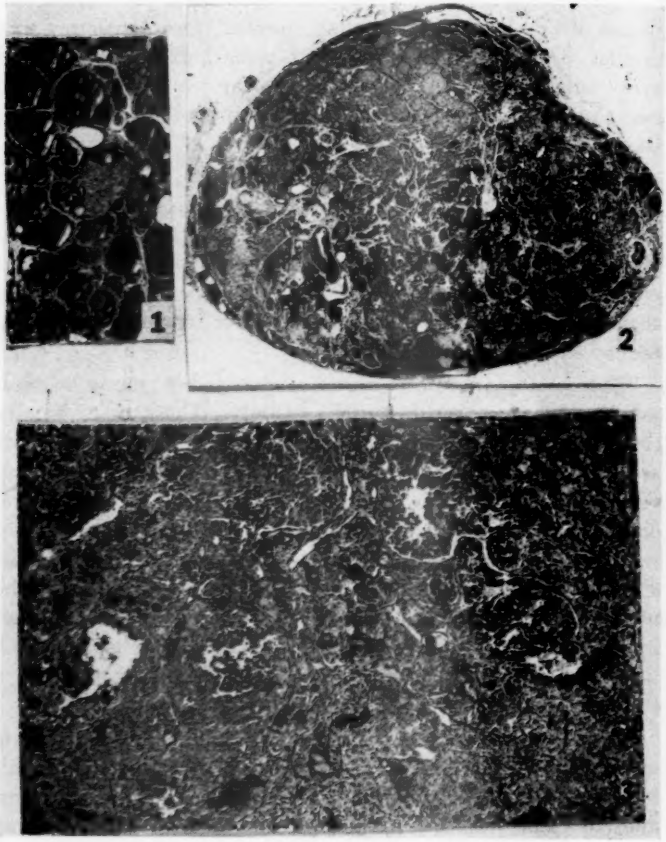

PLATE I. THYROID

FIG. 1. Control female rat, about 24 months old. Small nest of meta-hyperplasia, typical for most control rate. x 63.

FIG. 2. Female rat, about 27 months old. Treated with progesterone, thyroid hormone and oestradiol B.B. Adenoma of nearly whole thyroid. x 24.

FIG. 3. Male rat, about 22 months old, injected with oestradiol B.B. Varying size, structure and disposition of the adenoma cells. In places—beginning of cyst formation. x 63.

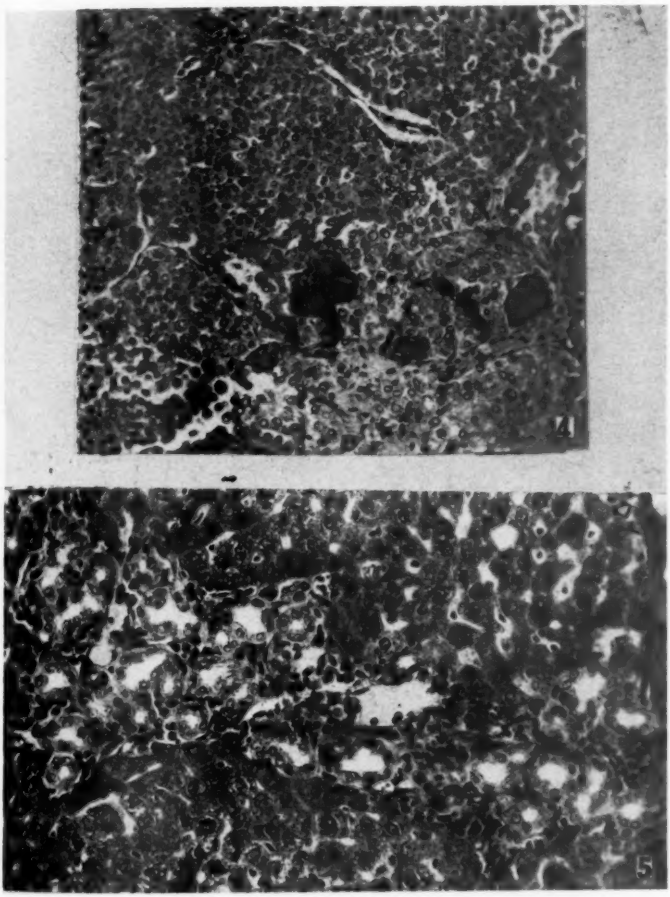

PLATE II. THYROID AND LIVER

FIG. 4. Thyroid adenoma in male rat, about 20 months old, treated with testosterone propionate, thyroid hormone and oestradiol B.B. Varying size and form of the cells and nuclei. x 140.

FIG. 5. Female rat, about 24 months old, treated with progesterone, thyroid hormone and oestradiol B.B. Adenoma-like hyperplasia of the smallest bile ducts. x 225.

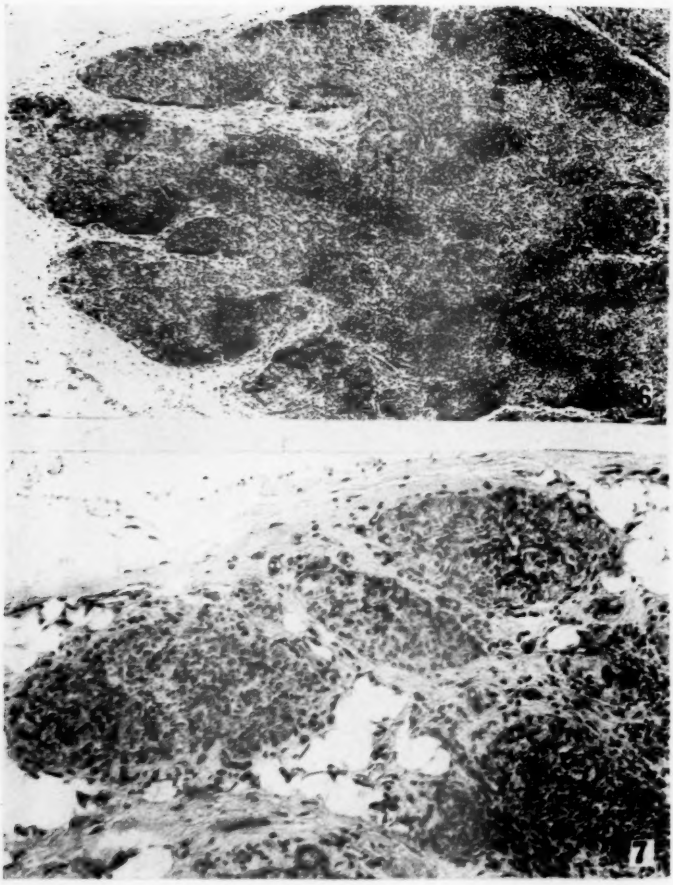

PLATE III. THYMUS

FIG. 6. Female rat, about 27 months old, treated with androsterone, progesterone, oestradiol B.B. and thyroid hormone. Spreading adenoma-like growth of thymic epithelial cells (gray) between remnants of normal thymic tissue (with small black nuclei). x 45.

FIG. 7. Male rat treated with testosterone propionate, oestradiol B.B. and thyroid hormone, about 20 months old. Normal thymic tissue atrophied (cells with small black round nuclei). Epithelial adenomatous cells—larger cells with larger gray nuclei of irregular shape. x 140.

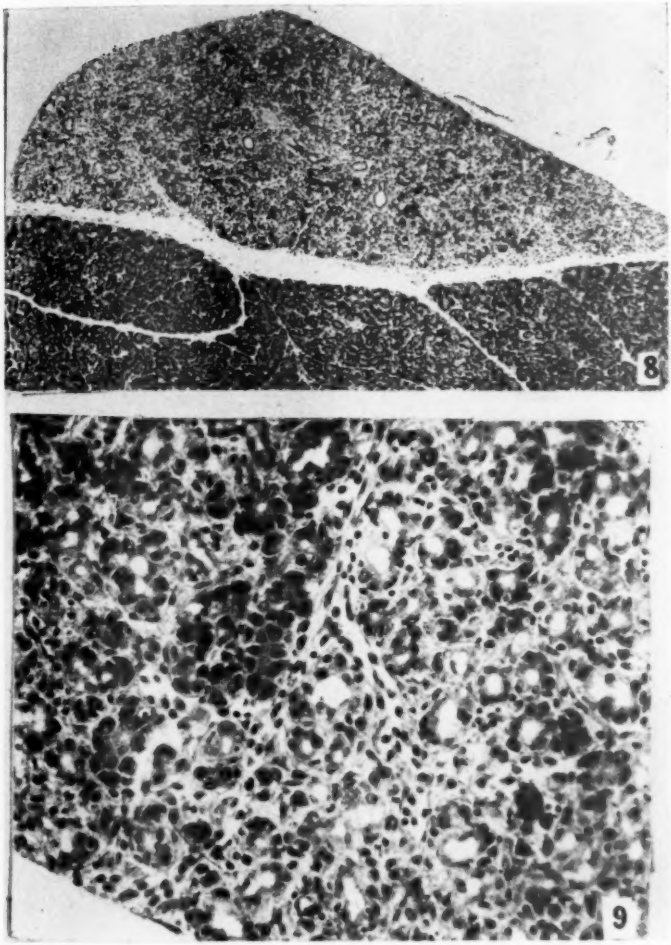

PLATE IV. PANCREAS

FIG. 8. Female rat, about 24 months old, treated with oestradiol B.B., progesterone and thyroid hormone. Adenomatous growth (gray lobule) above normal pancreatic tissue (acini with typical dark, haematoxylinophilic edges). x 45.

FIG. 9. The same rat as in fig. 8. Hyper-metaplasia of exocrine acini, with formation of large lumina, abnormal structure and, in some places, disorganized growth of the cells. x 225.

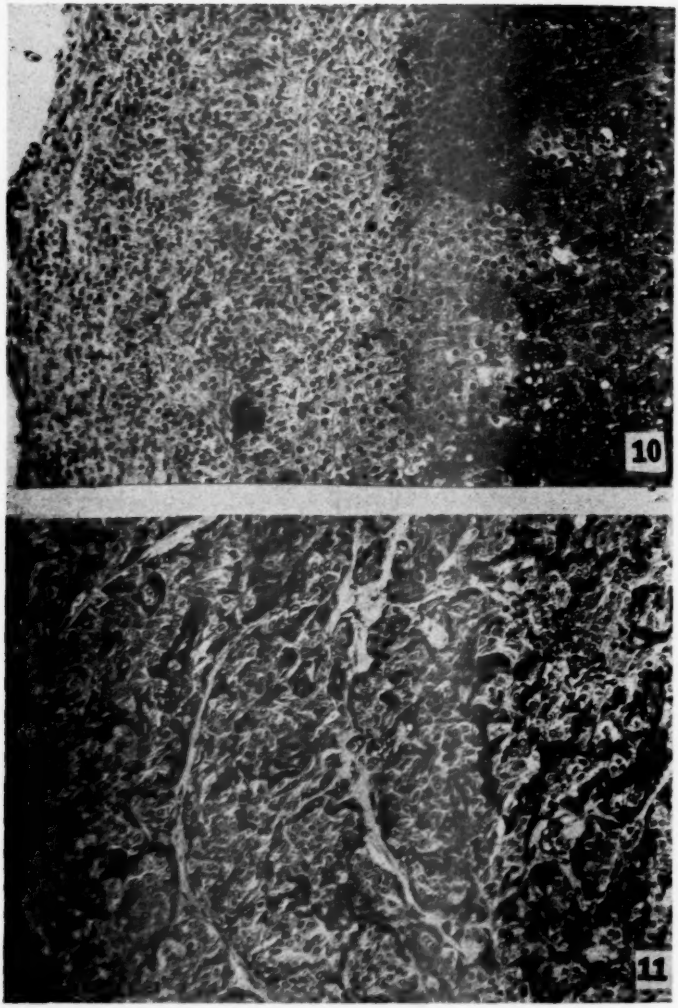

PLATE V. ADRENAL AND PARATHYROID

FIG. 10. Male rat, about 20 months old, treated with thyroid hormone. Adenoma (light area on left of photograph) originated from glomerulosa cells and spread over the adrenal as an extracapsular tumour. The latter, breaking through the adrenal capsule in several places, invaded zonae glomerulosa and fasciculata. These zones are normal at the top right of the photograph, but become meta-hyperplastic in the lower part, where they are invaded by the tumour. x 140.

FIG. 11. Female rat, about 27 months old, treated with progesterone, thyroid hormone and oestradiol B.B. Typical parathyroid adenoma, consisting of two kinds of cells: groups of "water-clear" cells between ribbons and nests of dark cells. x 140.

CERTAIN ASPECTS OF TUMOR ENZYMOLOGY

ALTON MEISTER*

Enzymologists have approached the problem of neoplastic disease from two general directions. One approach has led to the investigation of the enzymatic properties of tumors as compared to normal tissues, and the other to a study of the effects of neoplasia on the enzymatic behavior of the tumor-bearing host. The classical work of Warburg (1) which emphasized the high glycolytic capacity of tumors, represented the first comprehensive attempt to characterize the biochemical properties of tumors. More recently the studies of Greenstein (2) on a large number of enzyme systems in a variety of normal and neoplastic tissues have provided a basis for certain generalizations concerning the enzymatic activity of tumors. It was found that the range of activity of each enzyme was much narrower among tumors than among normal tissues. The uniformity of the enzymatic properties of tumors contrasted markedly with the wide range of activity in various normal tissues and the values for tumors were within the range of the normal tissues. The neoplastic transformation was associated with the disappearance of specific enzymatic functions, although occasionally some vestige of the original tissue of origin remained. Thus urea synthesis could not be demonstrated in hepatomas, while some hepatomas retained specific hepatic functions such as α-keto acid activated glutaminase and cystine desulfurase. It is of interest that these conclusions are entirely compatible with those of Williams and his collaborators (3–6) on the vitamin content of tumors. The B vitamin and ascorbic acid content is more uniform in tumors than in normal tissues. The relationships between enzyme activity and the vitamins is of course well known.

The data that have thus far been accumulated indicate that certain enzyme systems such as those associated with oxidative phases of metabolism fall in the lower part of the range of

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activity of normal tissues whereas other systems such as those associated with glycolysis and the metabolism of certain peptides are generally higher in tumors than in most normal tissues. Some enzyme systems notably those responsible for the dephosphorylation, deamination, and depolymerization of nucleic acids, arginase, and lactic dehydrogenase fall in the middle of the activity of normal tissues. Those engaged in studying tumor enzymology have first to determine the level of activity of various systems in tumors as compared to normal tissues. As more information becomes available the possibility of evaluating the relationships between the various systems will become more apparent. The acquisition of more knowledge of the enzymatic behavior of tumors frequently necessitates studies of fundamental biochemical reactions which may be useful in the study of tumors.

I wish to present for your consideration two problems in enzymology which are currently being studied in our laboratory. The first is the metabolism of certain keto acids by tumor and normal tissues, and the second the effect of neoplasia on the liver catalase activity of the tumor-bearing host.

I. *The Metabolism of Certain Keto Acids*

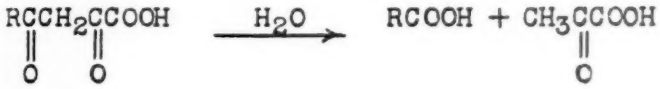
Several years ago it was observed by Greenstein and Carter (7) that the desamidation of glutamine by rat liver extracts was accelerated by pyruvic acid. A similar but less marked effect was observed with extracts of rat hepatoma. We have subsequently found that a wide variety of α-keto acids and α, γ-diketo acids accelerate glutamine desamidation in rat liver. However, as shown in Table 1, although α, γ-diketovaleric acid (acetopyruvate) and pyruvate accelerate glutamine desamidation to about the same extent in rat liver, acceleration was produced in extracts of hepatoma by pyruvate but not by α, γ-diketovalerate. Study of this interesting difference in the behavior of liver and hepatoma revealed that liver possessed an enzyme system capable of hydrolyzing α, γ-diketo acids to pyruvate and the corresponding fatty acid (8) in accordance with equation I. Of the normal tissues studied this reaction was found to occur at an appreciable rate only in liver and

TABLE 1

Effect of α, μDiketovalerate and Pyruvate on the Desamidation of Glutamine (8)*

Tissue	Keto Acid	Ammonia Formed (micromoles)	Per Cent Increase
Rat Liver	None	3.98	....
Rat Liver	Pyruvic	10.6	171
Rat Liver	Diketovaleric	10.7	169
Primary Rat Hepatoma	None	2.43	....
Primary Rat Hepatoma	Pyruvic	3.72	53.1
Primary Rat Hepatoma	Diketovaleric	2.41	0

*Digests consisted of 1 cc. tissue homogenate (333 mg.); 1 cc. veronal buffer at pH 7.0; 1 cc. glutamine (14 micromoles); 1 cc. water or keto acid (28 micromoles). Incubated 4 hours at 37°.



kidney. The reaction was followed by estimation of the pyruvate or fatty acid formed, or by the disappearance of the characteristic ultraviolet absorption curve of α, γ-diketo acids, which exhibits a maximum at 2950 A in alkali. A wide variety of α, γ-diketo acids was hydrolyzed under these conditions. Primary rat hepatoma was found to possess only a negligible hydrolytic activity towards α, γ-diketo acids and fetal rat liver also exhibited a somewhat decreased activity (9) (Figure 1). Thus we may conclude that the α, γ-diketo acids are active in accelerating glutamine desamidation only if they can be hydrolyzed and that the failure of α, γ-diketovalerate to increase glutamine desamidation in extracts of primary rat hepatoma is due to the low diketo acid C-acylase activity of this tissue. Transplanted mouse hepatomas also exhibit a decreased hydrolytic activity towards α, γ-diketo acids, although the reduction in activity is not as marked as in the case of the primary rat hepatoma. The relative rates of hydrolysis of the various diketo acids of this series are approximately the same in mouse liver and in the transplanted hepatoma (Table 2). The livers of animals bearing transplanted hepatomas possess normal levels of activity.

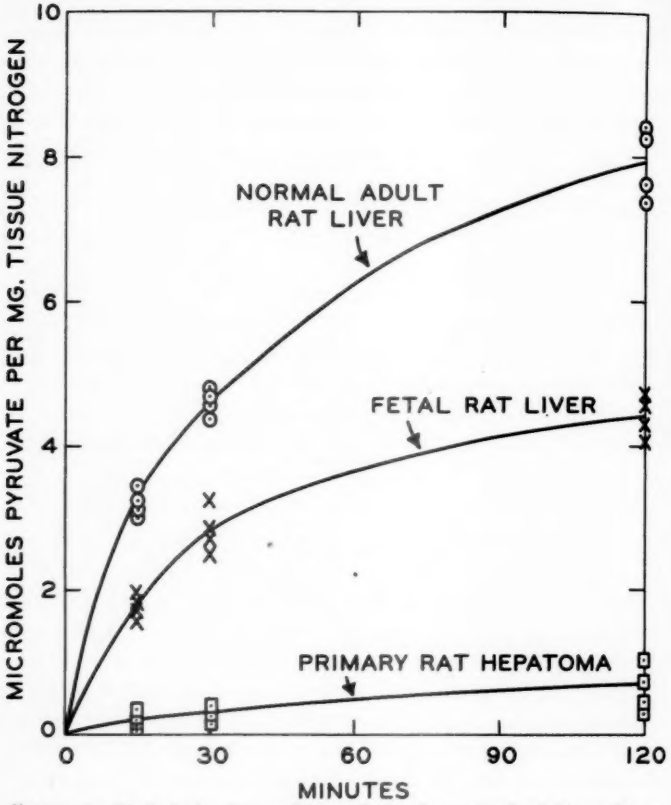


FIGURE 1. Hydrolysis of α, μ-diketovalerate by normal adult rat liver, fetal liver, and primary rat hepatoma (9).

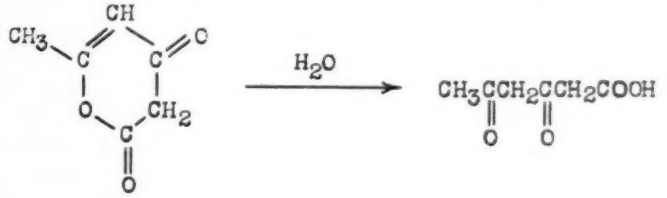
It seemed of interest to extend these studies to β, δ-diketo acids especially since such compounds have been suggested as intermediates in fatty acid oxidation according to the multiple alternate oxidation hypothesis. When β, δ-diketocaproic acid or its stable unsaturated δ-lactone was incubated with homogenates of liver or kidney the characteristic ultraviolet absorption curves of these substrates disappeared and acetoacetate and acetate were formed (10, 11). The mechanism of the enzymatic degradation of δ-lactone of β, δ-diketocaproic acid was found to involve two consecutive enzymatic steps described in equations II and III.

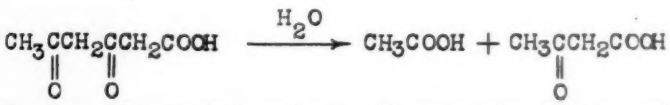
TABLE 2

Hydrolysis of 2,4-Diketo Acids by Normal Mouse Liver and Mouse Hepatoma 112B* (9).

Substrate	pH	Micromoles pyruvate formed per hour per milligram tissue nitrogen. Normal Liver	Hepatoma
2,4-Diketovalerate	7.3	20.8	6.88
2,4-Diketohexanoate	7.6	36.4	10.7
2,4-Diketoheptanoate	7.6	9.12	2.02
2,4-Diketooctanoate	8.3	5.66	2.24
2,4-Diketononanoate	8.3	9.50	2.92
2,4-Diketocaprate	8.3	8.44	2.54
2,4-Diketoundecylate	8.3	6.18	1.87
2,4-Diketo-4-phenylbutyrate	7.8	.379	.088

*Data were obtained with 0.5 cc. tissue homogenate and 0.5 cc. of 0.15M borate buffer containing 34 micromoles of substrate. Aliphatic substrates incubated for 5 minutes; 2,4-diketo-phenylbutyrate incubated for 1 hour.





Reaction III was independently discovered by Witter and Stotz (12). The enzyme responsible for the opening of the lactone ring is present in liver, kidney, and hepatoma and is associated with the submicroscopic particles of these tissues, while the enzyme which hydrolyzes the free diketo acid to acetic and acetoacetic acids is soluble. The relative rates of opening of the lactone and splitting of the free diketo acid were determined in normal and neoplastic hepatic tissues (13) (Table 3). Although the hydrolysis of the lactone by mouse liver occurs at approximately one-fifth of the rate of that observed with rat liver, the values for rat and mouse hepatomas are essentially the same. This is another example of the uniformity of the enzyme activity of tumors. The hydrolysis of the free diketo acid occurs approximately three times faster in liver than in hepatoma

TABLE 3

Metabolism of β, δ-Diketocaproic Acide and its δ-Lactone by Hepatoma and Normal Liver* (13).

Tissue	Lactonase Activity	Hydrolysis of Free Diketo Acid
Normal Mouse Liver	239	2.79
Hepatoma 112 B	251	.905
Hepatoma 98/15	191	.893
Hepatoma (azotoluene-induced)	290	1.11
Normal Rat Liver	1130	2.64
Primary Rat Hepatoma	208	.970

*Values for lactonase are expressed in terms of kx 10^6 per mg. homogenate nitrogen. Values for hydrolysis of free diketo acid are expressed in terms of micromoles of diketo acid metabolized per mg. homogenate nitrogen per hour.

of both species. In the course of the metabolism of the lactone by hepatoma, there is therefore less accumulation of free diketo acid than occurs with rat liver because the neoplastic transformation results in a greater decrease of lactonase activity than of diketo acid-hydrolyzing enzyme.

Studies on the elucidation of the function of the diketo acids in intermediary metabolism are being carried out. Considerable progress in this direction has recently been made by Ravdin and Crandall (14), who have demonstrated the formation of a β, δ-diketo acid in the course of the biological breakdown of homogentisic acid to acetoacetic acid. Studies in our laboratory on purified diketo acid C-acylase enzyme preparations suggest that α, γ-diketo and β, δ-diketo acids are hydrolyzed by the same enzyme. The hydrolysis of α, γ-diketocaproic acid occurs at a considerably faster rate than the corresponding β, δ-diketo acid (15). Further study of the diketo acid-hydrolyzing systems appears desirable.

Although the hydrolytic activity towards diketo acids appears to be limited to the liver, kidney, and hepatomas, it is possible that this class of substrates can be metabolized by other tissues through reductive and possibly other reactions. For instance we have recently found that α, γ-diketo acids are reduced in the presence of dihydrodiphosphopyridine nucleotide by muscle preparations (16, 17). This reaction may be followed spectrophotometrically by observation of the decrease of the absorption band of the reduced coenzyme at 3400 A and that of the diketo acid at 2900 A (at pH 7.1). The data indicate that equimolar

quantities of reduced coenzyme and diketo acid disappear (Figure 2). Although the position of the hydroxyl group has

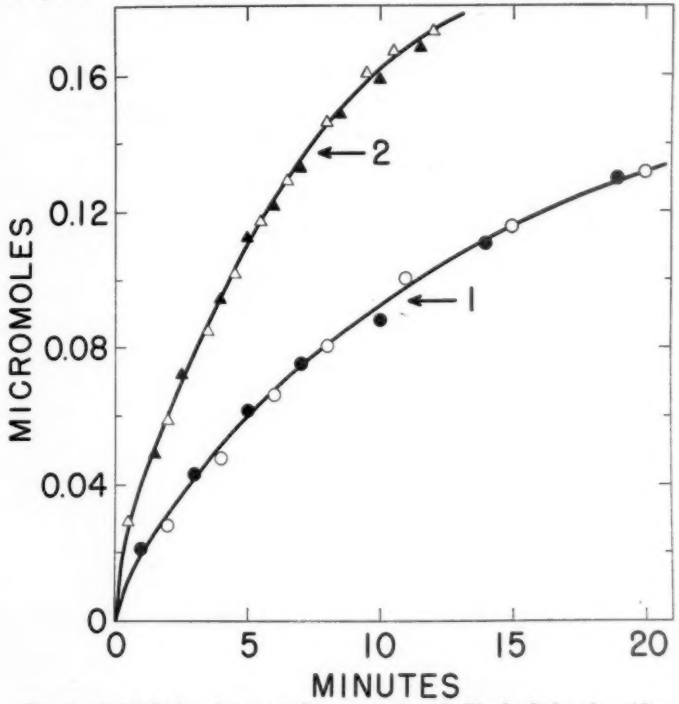


FIGURE 2. Relation between disappearance of dihydrodiphosphopridine nucleotide and α, μ-diketo acid (17). Curve 1: α, μ-diketovaleric acid with rabbit muscle preparation; Curve 2: α, μ-diketocapric acid with crystalline lactic dehydrogenase.

not been definitely established, it seems probable that the product of the reaction is the corresponding α-hydroxy-γ-keto acid in accordance with equation IV.

$$RCCH_2CCOOH + DPNH_2 \longrightarrow RCCH_2CHCOOH + DPN$$
$$\quad\;\|\;\;\|\qquad\qquad\qquad\qquad\quad\;\|\;\;|$$
$$\quad\;O\;\;O\qquad\qquad\qquad\qquad\quad\;O\;\;OH$$

Prior hydrolysis of the diketo acid to pyruvate was ruled out by the finding that no lactate is formed. It was found that this reaction is catalyzed by crystalline beef heart lactic dehydrogenase, and that this enzyme also catalyzes the reduction of a

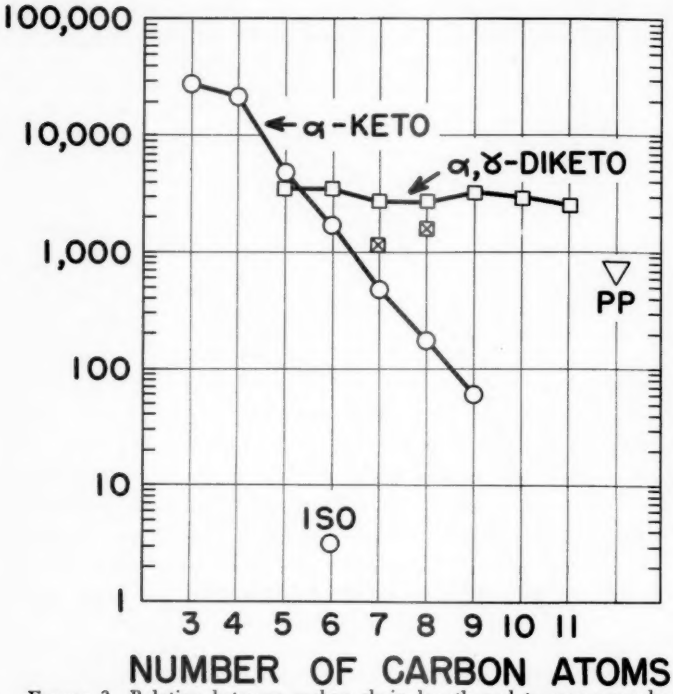


FIGURE 3. Relation between carbon chain length and turnover number of crystalline beef heart lactic dehydrogenase (17). Ordinate: (Logarithmic scale) Turnover number (moles of substrate reduced per 100,000 grams of enzyme at pH 7.2 and 26°). Abscissa: Number of carbon atoms in substrate molecule. Curves are drawn for the normal α-keto and α, μ-diketo acids. $\underline{[X]}$ = values for branched chain α, μ-diketo acids; ISO = α-ketoisocaproic acid; PP = phenylpyruvic acid.

number of α-keto acids (Figure 3). The reduction also proceeds with reduced triphosphopyridine nucleotide, although at a considerably slower rate. All of the α, γ-diketo acids tested except α, γ-diketo-δ-dimethylcaproic acid were reduced by the crystalline enzyme and reduced diphosphopyridine nucleotide. However, β, δ-diketocaproic and γ-ketovaleric acids were inactive in this system. The ability of tumors to catalyze the reduction of α-keto and α, γ-diketo acids is described in Table 4. The relative rates of reduction of the several keto acids are approximately the same for crystalline lactic dehydrogenase, mouse skeletal muscle, and the tumors studied.

TABLE 4

Reduction of α-Keto and α, μ-Diketo Acids* (18).

	Pyruvate	α-Keto Butyrate	α, μ-Diketo-valerate	Ratio
	(a)	(b)	(c)	(a):(b):(c)
Normal mouse skeletal muscle	990	578	103	1000:584:104
Transplanted rhabdomyosarcoma	273	160	26.0	1000:586:95
Transplanted gastric adenocarcinoma	347	228	36.5	1000:656:105
Spontaneous mammary adenocarcinoma	369	231	38.2	1000:626:104
Crystalline lactic dehydrogenase	167,000	95,600	19,800	1000:573:119

*Values expressed as moles x 10^{-8} of keto acid reduced per mg. of total nitrogen per minute at 26°.

An investigation of a variety of experimental tumors was carried out with pyruvate as substrate (18). The tumors exhibited relatively little variation as compared to the normal tissues studied (Figure 4). Although lung tumors exhibited a

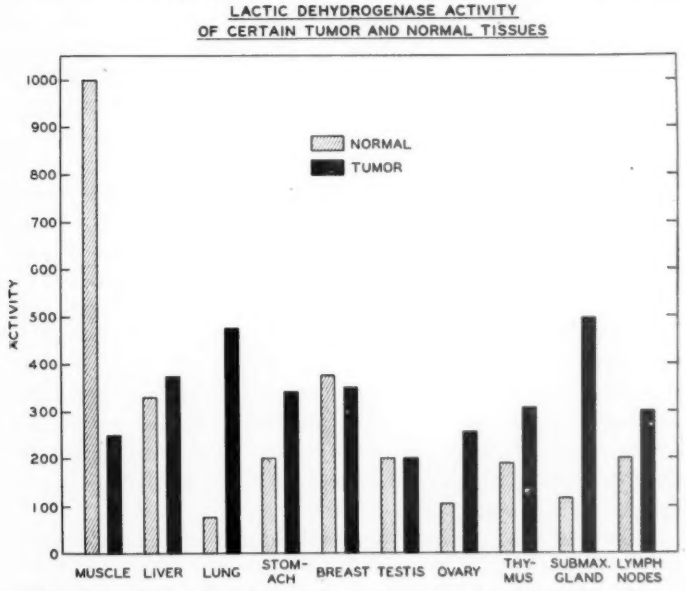


FIGURE 4. Lactic dehydrogenase activity of certain normal and neoplastic tissues of the mouse (18). Ordinate: Enzyme activity in terms of moles $\times 10^{-8}$ of pyruvate reduced per milligram of homogenate nitrogen per minute at 26°.

four- to ve-fold increase in activity over that of lung, the value for rhabdomyosarcoma was approximately one-fourth of that of skeletal muscle. In general the activity of tumors was appreciable as compared to normal tissues, and the level of lactic dehydrogenase activity in tumors may be said to be in the middle of the range of normal tissues. Although it is clear that our knowledge of the metabolism of the diketo acids is as yet incomplete, it would appear from the available data that the neoplastic transformation is associated with a considerable decrease in hydrolytic activity towards these compounds, while the capacity to reduce them in the presence of coenzyme is not appreciably altered.

II. *The Effect of Tumors on Liver Catalase Activity*

The lowered level of liver catalase in tumor-bearing animals was reported many years ago (19–22) and has more recently been the subject of considerable study by Greenstein and coworkers (23–27). These studies demonstrated that animals bearing rapidly growing tumors possessed markedly diminished liver catalase activity. The decrease in activity was progressive with growth of the tumor and the effect was reversed rapidly after extirpation or regression of the tumor. No decrease in liver catalase activity was observed in pregnant mice, mice bearing implants of embryonic tissue, or in mice infected with poliomyelitis virus, streptococcus, or staphlylococcus. A wide variety of tumors produced this phenomenon although it was absent with slow growing tumors and tumors smaller than 5% of the body weight. Weil-Malherbe and Shade (28) have confirmed these findings and showed that depression of liver catalase occurred whether a high- or low-protein diet was fed to the tumor-bearing animals. Recently Nakahara and Fukuoka (29) reported the isolation of a material from human neoplasms which, when injected into mice, produced a lowering of the liver catalase activity. These workers failed to find a similar compound in non-neoplastic tissues.

These observations have been extended in studies on experimental tumors by Dr. Robert Greenfield in our laboratory (30). A substance was obtained from transplanted mammary tumor C_3HBA and sarcoma 37, by extraction of the tumor with hot

water followed by precipitation with alcohol. By means of low-temperature alcohol fractionation a concentrated material was obtained which, when injected intraperitoneally in mice, produced a considerable lowering of the liver catalase activity. The effect is readily observable about 12 hours after injection, persists for two days, and disappears in approximately three days. The material is effective only *in vivo* and not *in vitro*. Studies of normal tissues have revealed the presence of much smaller quantities of a product which lowers liver catalase, although to a less marked extent than that observed with the material isolated from tumors. On the basis of the data now available, it is not possible to state whether the product obtained from normal tissues is the same as that obtained from tumors. The yields of active material from normal tissues have been very low, and large-scale isolation procedures will be necessary to pursue this aspect of the problem.

Whether or not the presence of the inhibitory compound is a specific characteristic of these tumors, it is a well-confirmed observation that animals bearing these tumors possess a markedly diminished liver catalase activity in comparison with normal animals of the same species. It has been found that injection of other proteins, buffer solutions, weak acids, and other fractions obtained from tumors, does not affect liver catalase. Although there may be other substances which inhibit liver catalase under these conditions, it seemed of importance to purify, isolate, and characterize the active product obtained from tumors. The most active material thus far obtained is a heat-stable, water-soluble white powder which contains about 16% nitrogen and behaves like a protein in solution. The activity is not lost by dialysis for 36 hours against water. Electrophoretic examination revealed a component comprising about 80% of the total substance, and several smaller components. When sedimented in the ultracentrifuge, 85% of the total material sedimented as a single component. Preliminary observations indicate that the molecular weight is in the range 15,000 to 40,000.

Further work on the purification and characterization of this tumor product is in progress, as well as investigations of its mode of action. The present evidence is compatible with the suggestion (2) that the lowered liver catalase of tumor-bearing

animals is due to a tumor product carried to the liver by the blood-stream. Although it is doubtful that this phenomenon can be applied to the problem of the early diagnosis of cancer at present, it may be possible to develop more sensitive procedures for the detection of this factor.

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TISSUE CULTURES OF CELLS FROM BODY FLUIDS*

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and G. G. Rose

In addition to its challenge in the field of diagnosis, the tissue culture method offers exceptional opportunities for a wide variety of researches on human pathological material. Cellular sources are readily obtainable as washings, collections of body fluids, biopsies, surgical specimens and tissues obtained at autopsy. Recent improvements in techniques have greatly simplified *in vitro* procedures. Among the most important practical advances are the availability of lyophilized plasma and embryonic extract from commercial sources, the use of antibiotics and the roller-tube method which provides an inexpensive, remarkably physiological, system for long-term culture work.

Human tissue material for experimental study are of considerable importance for certain types of investigations. This is especially true in the field of endocrinology where a wide variation in response is attributable to species differences to hormonal action. The problem is of particular interest in relation to malignancies involving the gonads. Research programs attempting to elucidate possible mechanisms of action, while intensely important in themselves, are usually limited to interpretations referable only to the animals utilized. Thus the brilliant work of Lipschütz and his collaborators (cf. Lipschütz, Yanine, Schwarz, Bruzzone, Acuña and Silberman, 1945; Iglesias and Lipschütz, 1946; and Iglesias and Lipschütz, 1947) on the relation of steroid hormones to fibromatoses begs extension to tissues of human origin. It is our belief that cells from human serous fluids offer a challenging opportunity in this direction.

Sources of Material

Fluid from the pleural and peritoneal cavities with various chemical and cellular compositions may be obtained from many

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different sources (Table I). While obviously most direct, the simple collection of fluid from the body cavities of experimental

TABLE I

Sources of Material for the Study of Pleural and Peritoneal Fluid

1. Collection of fluid
   - from laboratory animals
   - from humans during surgical procedures
2. Experimentally induced exudate-transudates
   - mineral oil
   - turpentine
   - diatomaceous earth
   - vascular interference
3. Thoracentesis and Paracentesis
   - non-malignant and
   - malignant series

animals or from the chest or abdomen of human subjects during exploratory operations is complicated by contamination with blood unless great precautions are taken especially in the handling of viscera. Standard procedures in experimental pathology, such as the use of peritoneal injections in rabbits of saline to produce cellular exudation (polymorphonuclear neutrophiles) or of mineral oil (for monocytes) as described by Wagoner and Custer (1932), may be found valuable. Maximow made extensive use of peritoneal exudates from rabbits following treatment with diatomaceous earth and with vital dyes (cf. Maximow and Bloom, 1931). Exudates produced by the injection of turpentine in the pleural cavity of dogs have been shown by Menkin (1940, 1948) to produce a remarkably valuable preparation for the study of the mechanism of inflamation. Vascular interference, especially of the type very successfully developed by McKee, Schloerb, Schilling, Tishkoff and Whipple (1948), is of prime importance in the study of the mechanism of fluid formation and drainage.

Of inestimable value to the researcher is the wealth of pathological material available by thoracentesis and paracentesis from human subjects. A wide spectrum of physico-chemical and cellular combinations are available in fluid accumulations resulting from non-malignant and malignant processes. The tables for the classification of these fluids in volumes by

Bodansky (1939), Wells (1925) and Yater (1942) have been found especially useful.

Laboratory Procedures (Table II)

TABLE II

Laboratory Procedures Employed in the Study of Pleural and Peritoneal Fluid

A. *Physico-chemical*

1. Physical Features
   - Quantity and relative rate of formation
   - Appearance (color, viscosity, etc.)
   - Sp. gr.
2. Chemical Features
   - pH (potentiometric)
   - Inorganic and pyro P (Fiske-Subbarow)
   - Free and protein tyrosine and tryptophane (Folin-Ciocalteu)
   - Protein and non-protein N (Kjeldahl & Nesslerization)
   - Glucose (Folin-Wu)
   - Cholesterol (Buckhard-Liebermann)
   - Gmelin (bile pigments)
   - Mucin (as released tyrosine after alkaline hydrolysis of the glucoprotein((Glass & Boyd)

   Enzymes
   - Adenosine triphosphatase (Van Potter)
   - Acid phosphatase (Gutman-Gutman)
   - Alkaline phosphatase (Bodansky-Bodansky)
   - Proteolysis (release of tryptophane and tyrosine in 3 hrs. incubation at 37.8°C. at pH 5.0)

B. *Cytological*

1. Cellular Composition
   - Differential count of "natural" components
   - Description of pathological elements
2. Behavior in Culture (hanging-drop andd roller-tube)
   - "Modulations" of various cell types
   - Mitotic and migratory activity
   - Pattern of cellular associations
3. Experimental modification of the environment
   - Method of seeding
     - Amount of cells used
     - Use of plasma clot versus absence of clot
   - Basic nutritional factors
     - Use of autologous, homologous or heterologous serum versus ascitic fluid
   - Special chemical factors
     - Additional vitamins, amino acids, etc.
     - Sterols and evocators
     - Carcinogens
     - Quinones, N mustards, di-aminopurines
     - Other drugs

It is obvious that the value of laboratory studies on body fluids is determined by the degree of clinical co-operation which can be effected. The establishment of the diagnosis, preferably by post-mortem examination and the analysis of records of fluid accumulations with special reference to procedures such as X-ray therapy during the course of the disease, will decide whether or not the bulk of many hours invested in chemical and biological studies can lead to useful conclusions. Since much of the most valuable material in these researches derives from chronically ill, old patients having a long downward course, few are retained in much needed and expensive hospital beds for thoroughgoing studies.

The appearance of gross blood in fluid collections complicates chemical procedures and usually is a major barrier in the preparation of tissue cultures. Such specimens are usually not centrifuged but allowed to stand for 1–2 hours. The sediment is collected with a pipette for further concentration with light centrifugation. Using this procedure, the larger masses of material which settle readily may be made available for culture without an undue amount of accompanying erythrocytes. Specimens showing a large amount of bile, as evidenced by a greenish color, usually yield poor results in tissue culture. The washing of cellular sediments from such specimens and their cultivation in medium not containing autologus fluid is indicated.

Fluids having a specific gravity of more than 1.018 are generally considered to be *exudates*, while those below 1.015 are *transudates*. The determination of the specific gravity, therefore, is a simple, valuable part of the examination.

The interpretation of various quantities of chemical constituents in relation to pathological processes can be meaningful only after a statistically significant number of analyses have been performed. As yet, our series of cases with verified diagnoses is insufficient to do more than to sketch certain broad trends in limited areas of the study. Routine analyses are being performed for the inorganic and pyro P, free and protein tyrosine and tryptophane, protein and non-protein nitrogen, cholesterol, glucose, bile pigments and mucin.

As far as enzymes are concerned, the adenosine triphosphatase has not been found in some 20 specimens of ascitic fluid and, therefore, this test has been abandoned in favor of more promising analyses. Acid phosphatase is regularly present in both pleural and ascitic fluid while alkaline phosphatase was not found in 4 out of 10 pathological ascitic fluids. This remains unexplained at present.

Proteolytic activity was demonstrated in all of 5 pleural fluids which have been studied; negative in 2 specimens of hydrocoele fluid and negative in 9 out of 10 ascitic fluids. The exception was material obtained from a patient with a diagnosis of myxoperitoneum which was probably malignant.

The presence of enzymes in pleural and ascitic fluid may be related to the possibility of free exchange between the plasma and the serous pools. The demonstration of the movement of C^{14}-labeled lysine in dogs with experimental ascites by McKee, Wilt, Hyatt and Whipple (1950) gives evidence for this interpretation. Further studies on the problem in terms of the Donnan equilibrium in ascitic fluid, as suggested by the work of Bálint and Benkö (1947), are under way.

As is well-known, the free cells in serous exudates, under physiological conditions, include macrophages showing a marked selective capacity for the ingestion of particulate wastes and for the storage of vital dyes, desquamated mesothelial cells, small lymphocytes and occasional neutrophilic and eosinophilic leucocytes. In tissue cultures, polymorphonuclear elements generally disintegrate within 48–96 hours while all four of the remaining cell types are believed to transform into spindle-shaped elements. Continuous cinematographic records of these changes in all species, together with a study of possible acellular fibrillar organization associated with each cell, are greatly to be desired.

As in the case of quantitative chemical studies, a general description of the cellular elements in body fluids associated with various clinical entities must await further study. Description of findings in cultures developed from a limited number of observations have been reported (Coman, 1943; Ivers and Pomerat, 1947; and Ivers, Pomerat and Neidhardt, 1948). The enormous numbers of spindle cells occasionally found to

develop in tissue cultures of cells from patients with a diagnosis of carcinomatosis peritonei probably cannot be accounted for as the derivatives of the "natural" components of the serous fluid but probably represent stromal elements associated with tumors. Evidence for transformations from epithelial to connective tissue cell types has not been clearly demonstrated in our material. We have had the opportunity to study the establishment of epithelial sheets from three specimens of ascitic and one specimen of pleural fluid. These were believed to have represented the carcinomatous elements involved.

Our experience with special patterns of cellular aggregation seen in tissue cultures of these materials leads us to the conclusion that not only is the description of the natural history of transformations or "modulations" of each cell types a prime objective in this effort, but that much can be learned by careful observations of the particular groupings of epithelial and non-epithelial elements. Not only in cultures of cells from ascitic and pleural fluid but in recent observations in our laboratory on brain tumors, patterns of cell arrangements have been observed which can hardly be thought of as undifferentiated cell cultures but rather as "tissue" cultures in the true sense. The outgrowth which was obtained resembled corresponding sections from the organs invaded with the malignant elements. The establishment of an epithelial sheet with its associated "stromal" reacting cells from a suspension of free cells offers a challenging opportunity for further studies, especially in relation to scirrhous carcinoma. Our observations support the frequent pleas by R. G. Harrison and H. B. Fell for the need of more intensive study of "organization" *in vitro*, as contrasted to the fetish of the "pure" culture of cells.

The free cells of body fluids provide many technical advantages for studies with the use of tissue culture methods. In addition to the variety of available types, these can be readily handled by concentration methods which may even permit segregation of various cell species. Tedious cutting of primary explants is by-passed and with the use of cell count procedures (Evans, Sanford, Shannon and Waltz, 1950), seeding of cultures often can be performed in a reproducible manner. Tissue culture workers have shown that an adequate number of cells

is essential for the establishment of a vigorous colony. A recent statement on this subject has been made by Earle, Sanford, Waltz and Shannon (1950).

In our own experience, it has been found that cells need not be imbedded in a plasma clot to assure the successful establishment of colonies. This is advantageous for experiments in which the presence of a plasma clot might interfere with the penetration of drugs or nutrients having a large molecular size. Our current practice consists of suspending the cell concentrate in a solution composed of 50% autogenous ascitic fluid, 45% Gey's solution and 5% embryonic extract. A sufficient quantity of penicillin is incorporated in the salt solution to make a final concentration of 1000 units per ml. The glassware utilized for such cultures must be especially well cleaned since adhesion of cells to the glass is markedly influenced by surface tension. A #1 cover glass measuring 12 x 50 mm. is introduced into a standard Pyrex test tube measuring 16 x 150 mm. From a 3 inch, 16 gauge needle attached to a 1 ml. tuberculin syringe, 0.25 ml. of the well-mixed cell suspension is deposited evenly on the cover glass which is maintained in a horizontal position. The tube is closed with a rubber stopper and is placed in a tray for incubation in the horizontal position (without rotation) for 48 to 72 hours. Following the addition of a suitable fluid nutrient, the cultures are rotated at 8 revolutions per hour.

It is well-known that the growth of epithelial cells in tissue culture is favored by temperatures of between 32 to 35° C. (*cf.*, Gey, Hanks and Barrett, 1948). In the decisions involved in planning experimental studies on cells from body fluids, the temperature factor should be considered. We have not, as yet, made a comprehensive study of this variable.

For purposes of studying X-ray effects, cover glasses are withdrawn from the roller tubes and placed in sets of 5 on the bottom of Petri dishes which serve as moist chambers. X-rays are applied directly to the cells on the upper surface of the cover slips. Penicillin in the medium mitigates against the risk of contamination during the exposure.

In the course of studies on cells from serous fluids as well as in the cultivation of elements from human adult tissues (Lewis, Pomerat and Ezell, 1949; Rose, Pomerat and Danes,

1949; and Pomerat, Ewalt, Snodgrass and Orr, 1950), it became evident that ascitic fluid provided an excellent nutrient supply. Current researches, with adult human cells from body fluids, epidermis, nasal mucosa and the brain, are attempting to provide quantitative evidence regarding the relative growth-promoting potentiality of autologous, homologous and heterologous serum, malignant and non-malignant ascitic and pleural fluids as compared with human umbilical cord serum. The latter is well accepted as one of the most useful nutrient sources available for tissue culture studies but it is probably more difficult to obtain in quantity than would be a suitable ascitic fluid. While exact measurements are not yet available, practical experience strongly indicates that ascitic fluid from patients with malignancies is rich in growth-promoting qualities. Experiments are in progress to locate the agent or agents responsible for this action.

Systematic studies on the effect of the addition of vitamins, special nutrients and drugs as suggested in Table II are being made. By way of illustrating one of our approaches, the procedure being utilized for studying possible changes in cellular activities which might be induced by chronic dosaging with high concentrations of various hormones will be described briefly.

One of the first serious obstacles encountered in dosaging tissue cultures with sterol preparations was the difficulty of incorporating the substances in a miscible solution. Oil preparations interfere with the optical properties of the cultures and are apt to complicate subsequent staining reactions. As a result of experience with the solution of "Antabuse" in connection with an unrelated study being conducted in the laboratory (Edwards, 1949), it was decided to use Tween 80 as a solvent for the various sterols. Preliminary to its use as a solvent, the minimal quantity required to prevent all outgrowth from explants of various species of chick embryonic cells as well as the quantity producing a minimal degree of injury was determined for Tween 80 utilizing methods established in this laboratory (*cf.*, Painter, Pomerat and Ezell, 1949). Toxicity

studies on Tween 80 will be published elsewhere. For the purpose of the present study, it suffices that no cellular injury was to be expected from Tween 80 at the doses utilized.

A nutrient fluid phase was prepared, utilizing the supernatant ascitic fluid from the specimen which had yielded the test cells. This consisted of:

Ascitic fluid	50%
Gey's solution	45%
EE #II (15%)	5%
Penicillin q.s.	1000 units/ml.

The nutrient was then divided into five moieties to be used for the study of experimental variables.

The general scheme for incorporating sterols into the nutrient medium consisted of dissolving the crystals in hot 95% ethyl alcohol and slowly adding the solution, under constant stirring, to hot Tween 80. The alcohol and remaining water were evaporated on a water bath. While the materials was still hot, double-distilled water was carefully added with constant stirring. The concentration of the stock solution and the amount of Tween 80 employed are given in the description of the various hormones which were studied in the first series of experiments. The addition of the hormones from stock solutions to the nutrient media was made slowly under constant stirring. Care was used to avoid denaturing effects in the medium by employing a "Mag Mix" (Precision Scientific Co.) system. All solutions, including the control, were stirred for 15 minutes at approximately the same rate of speed. Five experimental preparations were used.

I. ESTRONE (Schering) was used at 5 gamma/ml. of the nutrient medium from a stock solution containing 0.5 mg./ml. and 0.3 g./ml. of Tween 80.

II. PROGESTERONE (synthetic—Squibb) was used at 15 gamma/ml. of the nutrient and prepared from a stock consisting of 1 mg./ml. in 0.175 g./ml. Tween 80.

III. TESTOSTERONE (Squibb) was used at 5 gamma/ml. of the nutrient solution and from a stock containing 0.5 mg./ml. and 0.175 g./ml. of Tween 80.

IV. *TWEEN 80 CONTROL* consisted of adding a quantity of Tween 80 equal to that used for progesterone (maximal) from a stock solution containing 0.3 g./ml.

V. *CONTROL.* This contained no additions but was agitated with a glass sealed magnet bar in the same manner as used for the other preparations.

The evaluation of effects in these experiments is based on the deviation from results obtained under so-called "control" conditions. End-points to be observed include:

1. Differential death and persistence of particular species of cells.
2. Differences in the course of differentiation of particular cells (modulations).
3. Deviations in the establishment of aggregation patterns of similar or varieties of cell types.
4. Mitotic index (per cent of cells in division based on at least 5,000 cells).
5. Differential phase-count. The percentage of cells in prophase, metaphase, anaphase and telophase.
6. Differences in cellular components (mitochondria, granulation, nuclei, nucleoli, etc.) as seen with fixed and cinematographic records using phase-contrast microscopy.
7. Some attempt is being made to analyze the character of the nutrient fluid which is removed at regular intervals. Special attention is being given to mucin, enzyme content, etc.

Summary

The purpose of this report is to re-emphasize the value of utilizing human cellular material for experimental studies. The ease of handling and varieties of opportunities suggested by preliminary work on the physico-chemical and biological properties of pleural and peritoneal fluid is outlined. Suggestions are offered concerning technical procedures and experimental attacks on the fluid and cellular components of serous exudates.

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PROGRESS IN CANCER

C. P. Rhoades*

Judge Woodward, I would like to state at the outset that my introduction, though considerably inaccurate, is a tribute to the graciousness of Texan hospitality. I feel very much as my boss, Mr. Kettering, felt one morning when he came into the General Motors Research Laboratories to meet me there on another mission. He had come from New York; he came off the train somewhat the worse for overnight travel, and he remarked that he was awfully glad to leave New York and come back where he felt at home. He said in dealing with bankers in New York, they were only interested in going concerns; for his part he cared about coming concerns, like those down in Texas.

When our association with Mr. Kettering began, about five years ago, he asked me to sit down with him and discuss what we were trying to do. To define very clearly for ourselves and to his satisfaction, and that of others, that we had a clear conception of the goal we designed to attain and some understanding of the proceedings by which we proposed to attain this goal. That was rather illuminating because on thought it developed that we were not very clear, exactly where and how we were proceeding.

After a good deal of thought there was developed an outline of this problem, which we called a cancer problem. The concept was rather simple: it said in effect, let us examine the existing opinions about this problem. Let us assume that perhaps they are not correct. As you know this ability to analyze a problem is a distinguishing characteristic of the individual to whom I referred. The first question he asked was, why is it always said that "this cancer problem" must be very nearly insoluble because it must be as mysterious as the nature of life itself. Said he, "I cannot understand this statement; I have observed that a vast variety of medical problems which

*From the Sloan-Kettering Cancer Research Laboratories, New York City. These remarks introduced the Cancer Symposium held May 12, 1950, at the M. D. Anderson Hospital for Cancer Research, Houston.

seem to be most insoluble have yielded to able and intensive investigation, without knowing the nature of life itself, and I don't understand why this invasion by malignant cancerous cells should be regarded fundamentally as being different from the invasion of the body by parasites or by bacteria, both of which can be controlled." On careful thought some of us could see no reason to oppose this position.

So then we developed a very simplified theme based upon the fundamental concept that the ability of cells to change their characteristics and to vary in their relative ability to compete with their colleagues, or their parents, or their relatives, is one of the most standard phenomena which one encounters in biological life. It holds true all the way from the most minute forms of living organisms, the viruses, through the unicellular forms, the bacteria and larger organisms, though lower organized life, indeed to man himself, and men can become cancerous in relation to their neighbors as can cells. If that happens there is only one possible answer; one must destroy the malignant area, be it a bacterium, be it a parasite, or be it a human being, and there is no more fundamental observation.

If this be true then, how shall we face the cancer problem and what progress has been made? It is interesting that on my last call to Houston, which is some seven years ago, the phrase was, how should the scientists of the country proceed to solve this problem, and the phrase I have encountered today is, how soon do you think it will be solved. Now this represents a profound difference in thinking, and it is due to the fact that there have been profound advances in scientific knowledge and in technical procedure. If then we face this problem as a simple biological phenomenon of the change of organized biological units, cells, to become more aggressive than their neighbors, how shall we proceed?

First, is it true that if one destroys these malignant, variant, or mutated cells that one can cure the disease? Why, of course, it is true. Because for years and at a rapidly increasing rate surgeons and radiologists are curing cancer by destroying the aggressive and invasive cells. We can divide the problem into two major divisions. One, the attack upon cancer which still

remains sufficiently localized, so that it can be removed in toto without fatal injury to the host which harbors it, and the second is the attack upon cancer which for various reasons either late diagnosis or the nature of the malignant cell has so scattered widely throughout the body when it becomes recognized that we must devise new means for reaching it. We cannot remove it entirely by mechanical means, and some new means is required. Accepting then these two major divisions, how do we proceed to attack, in a better and more effective fashion, cancer while still localized?

There are two subdivisions to this attack, one attains by education, by organization, and by improved technical procedure through the search, more adequate methods for the early diagnosis of the disease. Great advances have been made, and indeed in some states to such a degree that the percentage of the patients coming under medical care with cancer in a curable form has doubled within the past five years. What better proof could be at hand? Secondly, once this cancer has come under medical care, how can we improve the procedures for its mechanical removal. They are improving very rapidly, so rapidly indeed that if one consults the figures of the Metropolitan Life Insurance Company or any other adequate actuarial agency, it is perfectly apparent that the death rate from cancer on what is called an age adjusted basis, that is, the percentage of the population of the given age, is dropping very materially and has been doing so for almost ten years. Indeed, for some anatomic sites of cancer the improvement, the lessening of that phase, is as great as 25%. There is adequate reason to believe that if methods now in existence were adequately employed on a sufficiently large scale this figure could be enormously increased, perhaps doubled.

An example may be considered. Cancer of the uterus kills about 10,000 women a year. Examination of the facts reveals that of the women dying of cancer of the neck of the uterus, the cervix, 50% die with the disease still localized to the pelvis, not scattered widely throughout the body. If then one could remove the pelvic contents surgically with subsequent health and useful life to the patient, one should be able to restore to useful and happy life, one-half of those who are now doomed

to a most unpleasant and prolonged terminal course. This has been undertaken in several clinics. It is fully possible. The proceedings are difficult, they are time-consuming, they require technical skill, but by their development and application it has been possible to return to useful life a substantial number of women who otherwise would have been doomed to a most unhappy event.

This is happening in other fields of cancer with an amazing speed, so that cure rates are jumping almost overnight. Now, why is this true? It is due to education, it is due to the willingness of surgeons and medical men to face the problem as a soluble one, and to devote their time and effort to achieving a difficult goal. It is due to the ability of the surgeon and the medical man and the X-ray man and the nurse and the technician to work together as a team toward a common end, for the sheer joy of the public service to be had in seeing this remarkable result achieved, and that is a sincere joy.

If, then, we accept the principle that new and more effective surgical procedures are coming very rapidly and are yielding higher cure rates, how shall we improve X-ray procedures where surgery is not justified? It is conventional to talk about higher voltage X-ray equipment, building a hotter and hotter fire with which to burn out and destroy the malignant and invasive cells, but new ideas of how to make more specific the injury only to the cancer cell and not to the normal ones are developing, and indeed in the past two years there have been developed through co-operative research of three universities and two industrial laboratories and one private research institution a variety of new X-ray tools.

X-ray is simply another form of light, and if it is properly broken apart will yield an invisible rainbow, exactly as visible light when impinged upon a prism will yield a rainbow. If we place in a beam of visible light so split a transparent object which will absorb one of the other colors there will appear upon the rainbow a dark spot due to the absorption of the particular wavelength represented by that color, and so if a red band is placed in the beam of light, red will no longer be red of the rainbow but will be a black vacant spot, and this simple principle can be applied to wavelengths of light in the

invisible areas of the spectrum all the way from ultra-violet to X-rays.

The development of X-ray tools capable of yielding, if you will, by analogy single color X-rays has revealed the possibility that different types of cells absorb specifically different wavelengths of X-rays. There is a possibility but no proof as yet that perhaps cancer cells will absorb certain wavelengths which will not be absorbed by normal cells, and therefore a greater preferential killing of the cancer than the normal cell could be exerted, and this is the simple key which we are all striving to achieve. My point is not that there has been made a great discovery, but there has been brought together in this common enterprise of a physical nature in a most happy and useful co-operation a variety of technical disciplines capable of operating together to attain a common end.

Now the attack on cancer is more difficult. One can view it very much as one would view the attack upon weeds in a field of grass. You can do the job of getting the weeds out of that field of grass in two ways. You can analyze the soil for its constituents, chemicals, and you can define the chemical qualities of the soil which allow the weeds to grow better than the grass, to be cancerous in relation to the normal or grass form. You do this practically and you send to the agricultural experiment station a sample of soil, and you can be told how to change the constituents of that soil so that the grass will grow better than the weeds and there will be no longer the weed, the analogue of cancer in the human organism.

We are doing this with human beings and we are analyzing the bodily soil which is the blood. We cannot analyze all the constituents of blood, but we can analyze certain of them which pertain intimately to the growth of cancer, and these are the constituents which are known as the sex and adrenal hormones, and steroids. To obtain this analysis extremely delicate methods have had to be devised. It has been necessary to devise procedures by which one could extract from the urine of normal individuals and those with cancer, and other diseases all of these hormones. Once extracted one had to devise methods of separating minute amounts of each from every other one. Having separated one from the other, one had

to have procedures by which one could define the nature of the particular hormone in enormously weak solution in a bottle. This was done by physical methods under which one could pass a beam of invisible light in the infrared spectrum, through the bottle, could amplify the energy exerted by this light and thereby could make the energy drive a pen which infringed upon a moving sheet of paper and you made the hormone write its own name, not in letters but in characteristic lines on the paper. These are just as specific and characteristic as the letters written by you when you sign a check, and then there could be set up a library of these signatures written by the chemicals themselves. Devoted women could be trained to recognize the signatures of their old friends, the known hormones, and to recognize new signatures of previously unknown compounds.

By the application of these procedures it is possible now to say that when cancer is present there is a consistent abnormality both in kind and amounts of the hormone manufactured and excreted by the human organism. It is also possible to draw the suggestion but not to advance complete proof that these changes antidate the appearance of the cancer. More evidence is needed, this conclusion is in accord with the best experimental evidence on the subject and suggests that the cancer cell requires a particular kind of soil under which it springs into being and continues its aggressive and destructive growth. If you modify this soil to restore normality you can stop the growth of the cancer cell, at least for a substantial period and in some the relatively few types of anatomic cancer. This can be done and is being done today. We are very hopeful that it will be done tomorrow much more adequately and on a much larger scale. There is no reason to suppose that this will not be the case.

In the course of this study it became necessary to prepare synthetically a large variety of these hormone compounds of a certain chemical type. In Texas Doctor Huffman has made a particularly notable contribution to this field, so that now we have on the shelf something over 200 different relatives of the natural hormones excreted by human beings. They have been coming to hand in adequate amounts, and they are

being administered to patients and to experimental animals. Interesting and important results are beginning to be achieved.

This represents the product of a vast amount of meticulous scientific labor, and a very distinguished advance toward practical clinical applications.

The second area is the control of disseminated or scattered cancer. We discussed the control of the soil which allows the cancer cell to spring into life and to grow, and its control by compounds now being prepared in the test tube by the accomplished synthetic chemist. The second area, again like grass and weeds, is based upon the demonstrative principle that one can devise chemicals which will kill one type of cell and not kill closely related cells. One example you all know, is that given by herbicides which will kill certain weeds and in proper concentration will not kill grass. Other examples are found in the efficacy of the sulfa drugs, penicillin, streptomycin, and analogous compounds which have the ability to kill invading and otherwise destructive bacteria without exerting serious deleterious effects upon the animal or human body as a whole.

The principle is completely accepted as far as invading bacteria or malaria parasites or spirochaetes are concerned, but it never has been accepted as far as cancer cells are concerned. We cannot see why it should not be accepted, and indeed every bit of evidence now coming to hand indicates that it should be accepted and that it exists as a principle. One may enumerate the points of this evidence. In the first place, there have been repeated demonstrations and one distinguished one here in the laboratory of Doctor Pomerat at Galveston, that under the proper circumstances cancer cells grow under artificial conditions in test tubes or their analogues in close contact with normal cells. When exposed to uniform concentration of certain chemicals the cancer cells can be destroyed and the normal cells will survive. This has been repeatedly demonstrated in a number of laboratories and is the key to the problem. Moreover in experimental animals more recently chemicals have been found which will injure seriously cancer cells without exerting a similar degree of injury upon normal cells. Thirdly, it is now possible to make cancer cells play most of

the tricks, that is to show most of the biological properties that are shown by bacteria, and other invading parasitic organisms. One example is bacteria if they are treated by one of the conventional bacteria killing chemicals, such as streptomycin and if they are not all killed will develop what are called antibiotic or in this instance streptomycin resistant strains which no longer are influenced adversely by this particular compound otherwise effective.

Precisely this phenomenon can be demonstrated for certain types of cancer cells in experimental animals and undoubtedly attains for cancer cells in human beings. It really has been known for a long time, but the pertinence of this whole clinical observation and this new experimental observation to the conceptual attack on the problem has not been recognized. It does tell us, in my opinion and that of others, that one can view the cancer cell as simply another type of invasive micro-organism and thereby can attain its killing by proper means if there is adequate research done by competent people. Every bit of evidence suggests it may well be true. Now if one wishes to kill one type of cell, be they bacteria or malaria parasites, or worms, or cancer cells, living in another organism one can go about it in two ways. One may analyze the two types of cells, the invader and the host, by any means you have at hand to define peculiar appetites and peculiar constituents which establish differences between the two types of cells. Such analyses are now under way in a variety of laboratories. They suggest very strongly that profound differences exist in appetites of normal and comparative cancer cells. The moment one can define such differences in appetites, the need for specific food constituents, one can make either poisonous or radioactive be preferred constituents and thereby destroy the cell which prefers that kind of diet, and leave the less greedy cell unharmed.

This is done today with radioactive iodine as concerns cancer of the thyroid gland where the cancer cells have a greater appetite for iodine in some instances but not in all, as compared to any other type of cell in the body. It is being done as compared to other types of diets preferred by cancer cells over normal cells, and Doctor Skipper in Birmingham at the

Southern Research Institute has made particularly notable contributions to this important topic.

Another way exists of going after this problem. One can simply try the various chemicals which one thinks might exert a preferential killing effect upon cancer cells, but one can only try them effectively if one has an exact method for measuring a delicate degree of the effect one wishes to exert. Such methods have now been developed. One can grow cancer cells on the developing chick embryo, and innoculate the egg with the chemical you wish to test. If you destroy the cancer and don't destroy the embryo, you have a compound that warrants further investigation. You can grow in test tubes cancer cells alongside their normal analogues. You can add to the medium in which they are growing proper concentrations of a test chemical and if the cancer cells are destroyed and not the normal ones, you have a hit and further study is desired. You may implant a variety of different forms of cancer in experimental animals and treat the animal.

A variety of compounds have now been found which will restrain substantially the growth of the cancer without considerable harm to the host animal. Indeed when one is dealing with very sensitive cancers in the experimental animal in some instances one may cure the cancer without harm to its host. Finally in human beings chemicals are now coming to hand which have a profound restraining effect by direct injury to the cancer cell without exerting an equal degree of injury to the other, the more normal cells of the body. These are not curative compounds, but they are compounds which tell us that better ones most certainly can be found.

It seems to many of us that these represent real advances. They will not only lead us to a better understanding of the nature of cellular tissues and of life itself but can be converted to practical advances in terms of prolongation of life if not cure indeed of forms of cancer not now curable. We have then good evidence that progress is being made. Clear evidence exists that one can remove safely forms of cancer not previously accessible to mechanical extirpation. Clear evidence is found that the cancer cell can be viewed simply as another invading and destructive organism, analogous to bacteria or

parasites. Clear evidence appears that one can exert preferentially upon this invader chemical injury by suitable compounds and more.

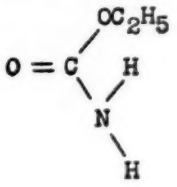
There has been mobilized in this activity a vast number of men of good will and of great competence who are acquiring rapidly the ability to work together with sincere devotion to a common goal. The remarks by Doctor Bertner represent in the highest way the type of thought, the noble wish to attain through devotion to public welfare that coming together of technical ability and men of character to achieve this goal and the greater glory of American science and American character. It does seem to me that here in Texas you are making most notable strides in this direction, and it is a great honor to us in New York to feel that we have made a contribution to this generous, unselfish and joyful enterprise.

INVESTIGATIONS ON THE MECHANISM OF THE ANTI-LEUKEMIC ACTION OF URETHAN EMPLOYING CARBON 14 COMPOUNDS*

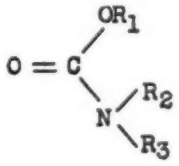
HOWARD E. SKIPPER

The very fact that so many cytologic, histopathologic, and biochemical responses have been observed in experiments with urethan complicates any attempt to select the systems involved in, or the site of the well-proven temporary anti-leukemic activity of the compound.

Urethan appears to be a highly specific molecule with regard to anti-leukemic action. Changes in the ester group, mono- or disubstitution at the nitrogen, or substitution of sulfur for the carbonyl or the ethoxy oxygen (see Figure 1) all destroy or lessen the anti-leukemic action of urethan in mice with chloroleukemia 1394 (1).



Urethan
(ethyl carbamate)



Substitution model

FIGURE 1

There is apperently no correlation between the anti-leukemic and the leukopenic action of carbamates (1, 2), but there is a correlation between the anti-leukemic and the carcinogenic activity (1, 3, 4, 5) and there is also a correlation between the leukopenic action observed in mice (2) and the inhibition of sea-urchin egg cleavage caused by substituted carbamates (6). These facts would seem to suggest that two basic mechanisms

*From the Southern Research Institute, Birmingham, Alabama. Presented at the Cancer Research Symposium, M. D. Anderson Hospital for Cancer Research, Houston, May 12, 1950.

might be involved in therapy with ethyl carbamate, one having to do with cell division and the other having to do with differentiation processes. The latter action perhaps is more closely related to the anti-cancer activity of the compound in question. Since differentiation is thought to be controlled by genes (composed largely of desoxyribose nucleic acid) and urethan is known to cause chromosome breaks, we have considered it important to direct certain of our studies toward possible relationships between the compound in question and nucleoprotein metabolism.

Experimental

In the present tracer investigations on the mechanism of action of urethan in cancer, emphasis has been placed on the following approaches:

1. Observation of the anatomical distribution of two of the carbon atoms of the urethan molecule (carbonyl and methylene—see Figure 2) in normal and neoplastic mice at various periods after injection.

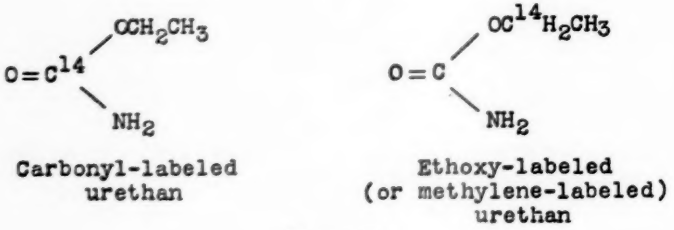


Figure 2

2. Studies on the rate of degradation and excretion of urethan as measured by expiration and excretion of active atoms of the compound. These studies were also carried out in normal and neoplastic animals.

3. Investigation of the fixation of urethan to important cell constituents.

This brief review will present experimental results in the order indicated above.

Anatomical Distribution of Carbon Atoms from Urethan in Normal and Neoplastic Mice

Since urethan causes an increase in the weight of the adrenals (7) (adrenalectomy renders rats more susceptible to leukemia; adrenal cortical and pituitary adrenotropic hormones retard development of the disease (7)), it was considered important to determine if urethan or portions of the compound concentrated in the adrenals or other endocrine organs. In view of the well-known leukopenic action of urethan, it also seemed pertinent to ascertain if there was any preferential concentration or fixation of carbon atoms of the molecule in the hematopoietic system.

Carbonyl-labeled urethan and ethoxy-labeled urethan were injected into normal and neoplastic mice and distribution data such as are presented in Tables I and II were obtained twenty-four hours later.

The total activities (per cent of total dosage injected) retained in all tissues by normal and neoplastic mice injected with labeled urethan are recorded in Table III.

TABLE 1

The Specific Activity* of Tissues from *Mice Injected with Carbonyl-Labeled Urethan (24 hours)*

Tissue	Normal Mice		Neoplastic Mice	
	No. 1	No. 2	Spontaneous Leukemia	Mammary Carcinoma
Blood	0.25	0.20	1.46	7.98
Spleen	0.23	0.10	1.01	2.04*
Adrenals	0.08	0.04	0.14	0.18
Kidney	0.19	0.13	1.13	2.72
Liver	0.30	0.31	0.83	1.68
Testes	0.15	0.09	1.03	...
Thymus	0.16	0.04	1.24	0.30
Heart	0.12	0.10	0.90	...
Lungs	0.14	0.13	0.92	1.23
Lymph nodes	0.10	0.12	0.87	1.12
Brain	0.18	0.06	0.84	1.86
Muscle	0.07	0.07	0.96	2.18
Jejunum	0.43	0.40	1.34	2.10
Bone (with marrow)	0.35	0.25	0.37	1.06
Tumor	...	...	...	4.35
Tumor fluid	...	...	...	5.06

*Specific activity in microcuries/mole of carbon.
Note: 2.0 microcuries of labeled urethan injected per mouse.

TABLE 2

The Specific Activity* of Tissues from
Mice Injected with Ethoxy-Labeled Urethan (24 hours)

Tissue	Normal Mice No. 1	No. 2	No. 3	Mammary Carcinoma
Blood	0.51	0.25	0.26	3.02
Spleen	0.77	0.93	0.48	2.21
Liver	0.58	0.56	0.50	1.30
Kidney	0.48	0.57	0.51	1.37
Lungs	0.52	0.67	0.60	1.52
Brain	0.31	0.30	0.32	0.76
Jejunum	1.30	1.71	0.86	1.79
Muscle	0.17	0.16	0.07	1.10
Long bone (with marrow)	0.30	0.27	0.21	0.74
Bone marrow	0.41	0.26	0.14	0.44
Adrenals	0.06	...	...	0.27
Testes	0.18	...	...	...
Thymus	0.08	...	...	0.82
Lymph nodes	0.11	...	...	0.10
Mammary tumor	...	...	...	1.74
Control mammary tissue	...	...	...	0.35

*Specific activity in microcuries/mole of carbon.

TABLE 3

The Per Cent of the Total Activity Retained by Normal and
Neoplastic Mice at 24 Hours After Injection of Labeled Urethan

Compound	Normal (Average)	Neoplastic Leukemic	Neoplastic Carcinomatous
Carbonyl-labeled urethan	1.3	10.9;10.2	32.7;46.0
$NaHC^{14}O_3$	1.3	0.87	...
Ethoxy-labeled urethan	6.2	...	36.0
Methylene-labeled ethyl alcohol	7.3	...	...

Rate and Mode of Degradation and Excretion of Urethan

Since on purely theoretical grounds one would assume that urethan might break down in the body according to the reaction presented in Figure 3, experiments have been carried out comparing the rate of exhalation of carbon 14 (as $C^{14}O_2$) after the injection of carbonyl-labeled urethan, ethoxy-labeled urethan, and the two probable carbon containing hydrolysis products of this compound, $NaHC^{14}O_3$ and methylene-labeled ethyl alcohol.

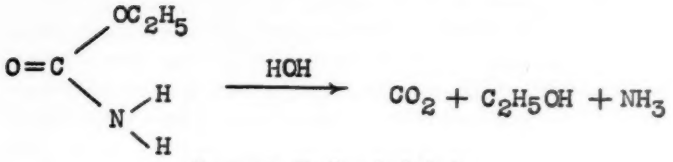


FIGURE 3. Urethan hydrolysis.

Rate of excretion curves and body distribution of carbon 14 following injection of the four labeled compounds mentioned (8) suggest strongly that a major portion of the observed fixation of carbon from carbonyl- and ethoxy-labeled urethan (presented in Tables I and II) is probably a result of fixation of the respective carbon atoms from the hydrolytic products of urethan, CO_2 and ethyl alcohol respectively (8).

The total body retention of carbon 14 following injection of the four compounds (on a percentage basis) is summarized in Table III.

The fact that urethan is degraded so much more rapidly in the body than can be accounted for by an uncatalyzed hydrolysis (at body temperature and pH) suggests that some enzyme must be involved in this hydrolysis.

Fixation of Urethan to Important Cell Constituents

Keeping in mind that any biological tracer experiments with carbon 14-labeled urethan must be corrected for fixation of the respective carbon containing hydrolysis products, we have attempted to learn more about the mechanism of action of urethan in relatively simple living systems (9). Selection of unfertilized eggs (resting cells) and fertilized eggs (dividing cells) from the sea urchin, *Tripneustes esculentus* has provided one comparison and sperm from a large sea cucumber another. This latter system was selected because of its chemical similarity to the isolated mammalian chromosome in that it is an isolated system composed largely of desoxyribose nucleohistone.

Using tracer techniques, it was found that fertilized and unfertilized urchin eggs fixed only a small amount of their accumulated urethan when exposed to non-inhibiting doses. There was no apparent preferential fixation of the carbonyl or the

methylene carbon atom of urethan in the eggs; and control experiments with $NaHC^{14}O_3$ and methylene-labeled ethyl alcohol provided fixation results as high or higher than were obtained with urethan carbon atoms. Sea-cucumber sperm, however, bound more of the urethan carbon than carbon from labeled bicarbonate or ethanol, Table IV.

TABLE 4

Fixation of Carbon 14 from Labeled Compounds by *Living Sea-Cucumber Sperm* (30-minute experiments)*

Compound	Total Activity Added to Sperm Suspension	Amount of Compound Added (mg)	Fixed Specific Activity† Vacuum Treated	Fixed Specific Activity† Dialyzed Against Cold Water
Experiment 1				
A. Carbonyl-labeled urethan	1.2	35.0	0.73	
B. $NaHC^{14}O_3$	2.0	17.2	0.08	
C. Ethoxy-labeled urethan	1.4	35.0	0.46	
D. Methylene-labeled ethanol	1.4	18.0	0.14	
Experiment 2				
A. Carbonyl-labeled urethan	1.2	35.0	0.18	0.022
B. $NaHC^{14}O_3$	2.0	17.2	0.02	0.008
C. Ethoxy-labeled urethan	1.4	35.0	0.24	0.099
D. Methylene-labeled ethanol	1.4	18.0	0.21	0.019

*25 ml of sperm suspended in sea water.
†Activities corrected for differences in total activity added to sperm. The free compounds were removed "in vacuo", acid added to the bicarbonate sample. Specific activities (μc/mole of carbon).

It was concluded from those experiments that urethan is accumulated within the dividing cell and exerts its effects on mitosis while free or loosely bound within the cell (inhibited eggs begin to divide again when urethan is washed away with fresh sea water). However, the specific fixation of carbon from two groups of the urethan molecule to sperm (high in desoxyribose nucleoprotein) suggests that a different mechanism may be involved in the well-known mutagenic, carcinogenic, and anti-cancer effects of this compound.

We have more recently found that urethan when given in rather large doses cuts down on the incorporation of formate carbon into nucleic acids while increasing the incorporation of formate carbon into tissue over and above that found in radioactive formate injected (otherwise untreated) mice. This latter observation conforms with observations of Hevesy (10). Preliminary data on this type of investigation are presented in Table V.

TABLE 5

Preliminary Studies on the Effect of Urethan on *Incorporation of Formate Carbon into Viscera Nucleic Acids*

Treatment	Urethan Dosage (mg/kg)	Specific Activity (μc/mole C) Viscera Homogenate	Combined Nucleic Acids
Control	....	8.48	57.6
Control	....	4.42	71.8
Urethan	1800 (1X)	7.36	45.4
Urethan	450 (6X)) 1800 (1X))	8.44	19.8

Note: Mice were injected with 1.4 μc of sodium formate and after six hours the viscera removed, homogenized, and assayed for activity. Nucleic acids isolated by the procedure of Schmidt and Tannhauser (11) were isolated from aliquots of the viscera homogenate and assayed for activity. In one experiment, urethan was injected at the LD50 (1800 mg/kg) immediately before $HC^{14}OONa$. In the second experiment urethan was injected at the level of 450 mg/kg on six successive days and with 1800 mg/kg on the seventh day immediately followed by labeled formate.

If one accepts the genetic theory that genes are extremely specific colloidal nucleohistone molecules of an enzymatic nature situated in orderly fashion along the thread-like chromosome, and that cancer is a mutation (a chemical or physical change in certain of these enzymatic nucleohistone molecules), then the present observations (fixation of urethan to sperm nucleohistone and inhibition of nucleic acid synthesis in mice following injection of urethan) become of interest. Certainly the specificity of these observed actions of urethan requires further study.

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ADRENALECTOMY IN PARABIOTIC RATS*

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Removal of endocrine organs which are maintained by the trophic stimulation of the anterior hypophysis usually induces hypersecretion of the related trophic hormone and its increased content in the hypophysis (Meyer, Biddulph and Finerty, 1946). Whereas most evidence supporting this concept has been derived from experiments involving castration, some adrenalectomy and thyroidectomy studies provide additional support (Finerty and Briseno-Castrejon, 1949; Griesbach and Purves, 1945).

The parabiosis technique provides a useful means of demonstrating variations in the amount of pituitary hormone secretion and has been utilized previously to indicate hypersecretion of adrenocorticotrophic hormone (ACTH) following adrenalectomy. Li and P'An (1940) found an average increase of nineteen per cent in the weight of the adrenals of a rat in parabiosis for fifteen days with an adrenalectomized partner, compared to adrenal weight of normal-normal parabionts. Houssay and Pinto (1944) showed a forty-two per cent increase in adrenal weight under similar conditions. Further support for this concept in other parabiotic experiments is that atrophy of the adrenals of an hypophysectomized rat was not prevented by parabiosis with an intact partner (Du Shane et al., 1935); whereas atrophy of adrenals of an hypophysectomized rat was prevented by parabiosis with an adrenalectomized partner (Jacobsohn, 1949).

Even though this evidence indicates that adrenalectomy in parabiosis is followed by hypersecretion of pituitary ACTH, similar response to bilateral adrenalectomy in single rats has not been demonstrated. Unilateral adrenalectomy experiments, however, do provide evidence that adrenal deficiency evokes heightened ACTH secretion (Finerty and Briseno-Castrejon,

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1949). Apparently any increase in pituitary activity following bilateral adrenalectomy is masked, or inhibited, by the inanition and the degenerate state of the animal (Reese, Koneff and Akimoto, 1939); a condition which is not present in parabiosis, or in unilateral adrenalectomy.

Since the conclusions derived from experiments on adrenalectomy in parabiosis are based upon scattered references utilizing few experimental animals the present experiments are presented as supporting evidence for the concepts that: 1) totally adrenalectomized rats will survive in good health in parabiosis with normal rats; 2) an increase in adrenal stimulating factors in circulating blood follows adrenalectomy.

Procedure

Littermate male rats of the Holtzman strain were united in parabiosis at the age of 31 days according to the method of Bunster and Meyer (1933), except that metal skin clips were used instead of silk sutures in closing the skin incisions. The right partners of experimental pairs were bilaterally adrenalevtomized at the time of parabiotic union. Twenty days later all pairs which survived (10 pairs normal-normal; 13 pairs normal-adrenalectomized) were sacrificed and body weight and ardenal weight were recorded.

Results and Discussion

Body weights and adrenal weights are shown in Table 1. The average weight of the adrenal glands of a male rat in parabiosis with another intact male rat is 29.6 mg. (24.6 mg./100 gm. body weight). Partners of male rats which had been adrenalectomized at the time of parabiotic union have adrenal glands weighing 42.3 mg. (35.9 mg./100 gm. body weight). It can be seen that an increase in forty-three per cent has occurred in response to the absence of adrenal glands in the partner (or forty-six per cent on an adrenal weight/body weight basis).

Marked variation in body weight of these animals may be partly explained by the unorthodox manner of living in parabiosis. Mortality rate was thirty to forty per cent in this experiment, with only those animals which survived for the entire

period of the experiment shown in the results. Most of the deaths could be attributed to "parabiosis intoxication," a condition of unknown etiology, characterized by hyperemia of one partner followed by dwarfing and death usually within a short period after symptoms appear (Schmidt, 1922). In a few pairs with poor surgical union, the adrenalectomized partner died with usual signs of adrenal insufficiency.

These experiments demonstrate that adrenalectomized rats, which ordinarily lose weight rapidly and dies within 10–12 days, will survive for a period of at least twenty days in good condition if they are in parabiosis with an intact rat. Not only do they survive, but they appear to thrive since the mean

TABLE 1

Body weights and adrenal weights of rats joined parabiotically to intact and adrenalectomized partners for a period of twenty days.

	Body wt. gm.	Adrenal wt. (absolute) mg.	Adrenal wt. (per 100 gm. body wt.) mg.
Left partners of intact-intact pairs			
	108	26.8	24.8
	92	19.6	21.3
	134	30.0	22.4
	135	32.0	23.7
	108	28.0	25.9
	160	30.6	19.1
	175	30.8	17.6
	118	32.6	27.6
	103	33.0	32.0
	102	32.2	31.5
Mean	123	29.6	24.6
Left partners of intact-adrenalectomized pairs			
	112	38.0	33.9
	134	35.4	26.4
	131	44.0	33.6
	125	35.6	28.5
	98	49.2	50.2
	99	32.2	32.5
	187	43.0	23.0
	135	42.8	31.7
	178	50.0	28.1
	199	44.2	22.2
	69	54.2	78.5
	100	43.0	43.0
	107	38.2	35.7
Mean	129	42.3	35.9

body weight of the adrenalectomized right partners in this experiment is 138 gm. in contrast to 129 gm. for the intact right partners. Appearance of the pelage and general well-being seemed better in the pairs in which one partner was adrenalectomized.

Whether maintenance of life and health of an adrenalectomized rat in parabiosis is effected by transmission of cortical hormones across the union to insure homeostasis or by crossing over of electrolytes to maintain adequate balance is not known.

Increased weight of adrenal glands of the partners of adrenalectomized rats indicated heightened activity of adrenotrophic mechanisms. This is presumably on the basis of increased adrenocorticotrophic secretion from the pituitary gland of the adrenalectomized partner. It must be considered, however, that the hypophysis of the intact partner might also undergo some hyperactivity due to the influence of blood exchange with an adrenal-deficient animal. Conceivably the presence of two pituitary glands affecting only one pair of adrenal glands could cause hypertrophy of the adrenals without an increase over normal secretion rate. Another added factor may be the disturbance of electrolyte balance which directly induces hypertrophy of the zona glomerulosa (Deane, Shaw and Greep, 1948).

Summary

Adrenalectomized rats will survive in good condition when in parabiotic union with normal rats.

Hypertrophy of the adrenal glands of an intact rat in parabiosis with an adrenalectomized rat suggests that there is increased adrenocorticotrophic secretion following adrenalectomy.

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VARIABILITY IN PROLONGED STORAGE OF ARSENIC IN RATS*

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Recognition of appreciable amounts of arsenic in tissues, secretions, nails or hair is generally taken to indicate that exogenous arsenic has been absorbed within a fairly recent period. The question of the amount of arsenic normally present in human beings has engaged the attention of many workers since Couerbe (1838), Orfila (1839/40) and Gautier (1899–1920). A Cited (Fordyce *et al.*, 1923) earlier reference to Couerbe (1834) contains no data on arsenic studies. The weight of the older literature leads to the conclusion that arsenic is not a normal or essential constituent in man, and is found to be present occasionally only through accidents of environment, diet or occupation, or by analytical errors. In 1905, Kunkel (1905) considered the literature at that time to be too voluminous and inaccurate to warrant a summary; only a part of the more pertinent references is given in the present report.

Camp and Gant (1949) found an As_2O_3 content of 0.25–0.88 microg./g. of 43 samples of normal human hair, confirming values of 0.10–0.70 microg./g. reported for 14 of 21 samples examined by Glaister (1947). Wührer (1937), who estimated the normal range as 5–50 microg./100 g., has reviewed the earlier literature. Content of As or As_2O_3 in microg./100 g. of normal human hair is noted by Heffter (1915) as 1000–5000, probably due to deficiencies of his analytical method; by Myers and Cornwell (1925) and Fordyce *et al.* (1923), as usually 5–180, with extremes of 0–10,600; by Billeter and Marfurt (1923) as 9.7 of As; Billeter (1924) as 12.3; Althausen and Gunther (1929), 80–160; van Itallie (1932), 10–40; Schwarz and Deckert (1931), 0–2800, in arsenic workers; Szép (1940), as 24–77; and Knecht and Dearden (1901), who found none

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in normal hair but 3000–10,000 in 3 patients who had ingested arsenic in contaminated beer or in therapeutic amounts as a drug. Young and Rice (1944) found 30 microg./100 g. of guinea pig hair, but cast doubt upon all earlier studies since they claim that nearly all of the arsenic in hair is easily extractable by mild solvents generally used to clean the hair before analysis, and note the impossibility of differentiating exterior and interior arsenic in the hair, extending the work of Smith and Hendry (1934). Schaaf (1944) found increased amounts of As in guinea pig hair after treatment of these animals repeatedly with $NaHAsO_4$. Schiff (1898) found traces of As in hair of dogs fed As, but none in untreated controls. Abel and Buttenberg (1899) demonstrated As in animal hair with their microbioassay for As. Young and Smith (1942) reported 320–440 microg./100 g. of hair from 2 cadavers exhumed after 9 years, with a content of 770 in the proximal hair of one.

Variation in their results is attributed by Camp and Gant (1949) to diet and environmental factors such as soot in the atmosphere, and was shown to be independent of tobacco smoking and the eating of sprayed fruit. In discussion of these data at the Detroit meeting of the Society for Pharmacology and Experimental Therapeutics, Camp stated that one very high As content of hair encountered was inexplicable except on the basis of a course of carbarsone taken by the patient some 3 years previously. Immediate urinary excretion of appreciable amounts of carbarsone after oral administration, noted by Chen et al. (1930), would make such prolonged storage unlikely. Similarly, results reported by Chance *et al.* (1945), of excretion studies of arsine oxides and arsonic acids, also cast doubt on the regularity of prolonged storage; arsonic acids are rapidly cleared by the kidney. Bailey and MacKay (1920) found no As in urine of patients given arsenical therapy for syphilis, 24–157 days after treatment. Since suitable material was present in this laboratory for a few tests of the variability in prolonged storage of As in rats treated with Na arsenite, the following tissue distribution studies were made.

Experimental

Five adult white rats were treated for 14 days with daily intraperitoneal doses of 10 mg./kg. of As_2O_3, given as diluted Fowler's solution. These rats were then maintained for 19 months on the usual laboratory diet, which contained 207 ± 19 microg./100 g., of As_2O_3; this amount is not excessive, in comparison with the As content of normal constituents of the human diet, as noted by Gautier (1903) and Bertrand (1903), but is above the tolerance for foods recommended by the Royal Commission (1903). Boos and Werby (1935) have noted the high As content of American foods.

One of the 5 treated rats died during the second month after treatment, while the remaining 4 appeared healthy throughout the 19 month period. Their body weight increased normally during the post-treatment period, and was 415–436 g. at its conclusion. Assays were made of As_2O_3 content of organs of the individual rats by the A. O. A. C. Official Gutzeit method. One of the rats was found to have a large cystic right kidney, with hypertrophy of the left kidney; as noted in Table I, this

TABLE 1

Arsenic Content of Rat Tissues

19 months after arsenic administration

	(Total amount as microg. of As_2O_3)				
	No. 1	2	3	4	Controls
Liver	..	23.9	3.4	1.7	2.5
Kidneys	0.78	21.1*	0.38	1.9	0.37
Hide	26.4	18.8	11.2	46.8	2.03
Lungs	22.8	11.2	0.2	15.2	1.1
Spleen	3.1	0.38	0.56	2.24	0.50
Heart	0.75	<0.1	0.56	0.85	1.1
Testes	0.91	<0.1	0.37	0.35	0.38
Al. Tract	10.1	6.6	6.4	16.4	3.3
Carcass	66.5	55.0	36.8	102.0	6.2

*Large cystic kidney.

rat had abnormally large amounts of As in the liver and kidneys, but not in the body as a whole. No other pathologic conditions were apparent grossly in any of the rats.

Arsenic content of the hair of the treated rats was extremely variable, although care was taken to sample the same areas of all animals; it was low in all instances. The first rat had 122 microg. % of As_2O_3; the second, a trace, well below the limit of precision of the test, representing about 10 microg. %; and the third and fourth, 30.2 and 28.3 respectively, which are within normal limits.

Of the other organs, all showed considerable variation. The hide (including some hair), the lungs and carcass showed the greatest storage of arsenic. For the liver, kidney and spleen, at least 2 of the 4 rats showed normal values. The heart and testes apparently do not store As for any appreciable time. The alimentary tract maintains a moderate increase above the normal As content, probably through its function of eliminating As; Clements *et al.* (1950) found high As content after parenteral administration, in acute studies.

Results were calculated also as concentrations of As_2O_3 in the various tissues. The above conclusions apply equally well to these data, even in the instance of the large cystic kidney, which still showed a markedly increased concentration of As despite its greatly increased weight of 19.6 g.

The total amount of As recovered in the individual rats represents about 0.2% of that given 19 months before assay, despite a cumulative dietary intake of possibly as much as 0.6 of that amount during this period.

Discussion

The chief characteristic of the above data relating to storage of As is their extreme variability. Analyses were controlled by estimations of As content of the reagents and comparisons were made of estimations of added As, carried through the entire procedure, and of As added to Gutzeit flasks. All digestions and distillations were carefully done, and the Gutzeit estimations were all made by the senior author, who has considerable experience with this method. Variations in results cannot be attributed to analytical errors.

One source of variability in As distribution has been studied by Gautier (1900), Guthmann and Grass (1933), Agduhr (1941) and Skanse (1941). Arsenic content of the blood varies

markedly with menstruation and pregnancy. Resistance to acute toxicity of As_2O_3 varies significantly with alteration in sexual function, as does also the amount of As stored in the skin. Storage of As probably depends to a major extent upon the content of sulfhydril compounds in tissues, which would affect the affinities of the tissues for As. Many physiological factors could influence the tissue sulfhydril content.

All of the rats used in the present study were adult males, maintained under similar conditions. The variability in As storage is most significant since the biological material was uniform.

Summary

A considerable variation of arsenic storage was found in 4 rats, 19 months after treatment with a course of intraperitoneal injections totalling 140 mg./kg. of As_2O_3. It is recommended that, for medicolegal purposes, quantitative estimations should be made, preferably, on several tissues in addition to hair, and that the possibility of prolonged storage must be considered even though such storage is not uniform.

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CORPORA LUTEA COUNTS AND THEIR RELATION TO THE NUMBERS OF EMBRYOS IN THE WILD NORWAY RAT*

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An opportunity to estimate intra-uterine mortality in a wild population of Norway rats *(Rattus norvegicus)* was presented by a histological study of the ovaries and a dissection of the uterine horns. The purpose of this paper is to present the data on the numbers of corpora lutea found in the ovaries and their correlation with the numbers of embryos recovered, and also to describe the criteria used to distinguish the different sets of corpora lutea in each pair of ovaries. An estimation of the frequency of estrus, as judged by the number of sets of corpora lutea present in the ovaries, was attempted to help explain seasonal change in pregnancy rate (Davis and Hall, 1950a).

Although there are a few reports on pregnancy rates in the wild Norway rat (Buxton, 1936), little well documented information on this species exists. Recent studies attempt elucidation of their reproductive pattern in the wild state. Perry (1945) studied sex-ratio of embryos, reproductive cycle in the male and female, and fertility in rats collected from several habitats in and around Liverpool and Warrington, England. Davis and Hall (1948; 1950a) reported on the seasonal reproductive condition in the male and the female wild Norway, as well as the pregnancy, litter, and embryo rate, lactation with and without pregnancy, and criteria of maturity in these rats from a uniform ecological habitat in Baltimore, Maryland.

Materials

The rats were caught in steel traps in an ecologically uniform habitat, the Baltimore poultry warehouses, at regular intervals over a continuous period of 13 months. They were

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brought to the laboratory and autospied as described by Davis and Hall (1950a).

For histological study of the ovaries, the organs were fixed in Bouin's fluid, stained *in toto* in cochineal, serially sectioned at 10 or 15 micra, spread on 2 x 3 inch slides, counterstained on the slides with cotton blue, mounted in Canada balsam and covered by plastic slide-covers 48 x 63 mm. A total number of 336 pairs of ovaries were so prepared and studied in this series.

Embryo counts were made from enlargements seen on the surface of uterine horns at the time of autopsy. These were checked after fixation when the uterine horns were dissected and the embryos exposed. Measurement of embryos was made in the fixed state after their dissection from the fetal membranes. The crown-rump dimension was measured. Embryos under 3 mm in length were not measured though their numbers were checked. Lactation was determined by expressing the mammary glands at the time of autopsy and noting the presence of a white secretion.

Sets of Corpora Lutea

Histologic studies of corpora lutea to date have been almost exclusively on the albino or laboratory rat and for the most part have concerned themselves with size (Weichert and Schurgast, 1942) or volume (Boling, 1942) of the gland. From the classic study by Long and Evans (1922) it was apparent that grouping of corpora lutea into their respective sets, corresponding to several cycles that occurred during the life of the oldest corpora lutea, would be difficult as these sets of corpora lutea were separated by only 4–5 day intervals. Furthermore the superimposition of new sets upon the existing structures which had not yet regressed would add to the difficulty of counting the number of corpora lutea from each set of ovulated follicles. These workers reported the presence of up to 50 corpora lutea in one ovary.

In our material morphological differences between sets of corpora lutea were distinctly apparent and in majority of the cases there were not as many sets as anticipated. This pointed to either a difference in the length of the estrous cycle in the

wild as compared to the laboratory Norway rat or, as suggested by Boling (1942), the rate and manner of regression of the corpora lutea may be different in various strains of rats. Differences in response of various strains of laboratory rats to corpus luteum formation under influence of the pituitary hormones has also been suggested (Pfeiffer, 1942). There are indeed several noted differences between the laboratory and the wild Norway rat such as size, appearance and response of the adrenal, size of the preputial glands, polyovuly, the presence of accessory corpora lutea and others (Hall, 1950).

The morphological criteria we used attempted only to differentiate between sets of corpora lutea as being "recent," "new," "old" or "remnants" of sets in each pair of ovaries. Corpora lutea designated as "recent" were newly forming structures often being Graafian follicles which had just discharged their ova; while those designated as "new" were already fully formed corpora. Differentiation between corpora was found to be not difficult as the majority of ovaries studied had only two quite easily distinguishable sets. Chronological ageing of the corpora lutea was not attempted.

Morphological Criteria Used to Distinguish Between Sets of Corpora Lutea

For convenience we shall start our description with the immature ovary. Here no corpora lutea are present, their absence being a criterion of immaturity. Varying numbers of primary and secondary follicles in the younger animals and in addition, increasing numbers of Graafian follicles in those approaching maturity, were apparent. In our material 17.9 percent of the pairs of ovaries examined were in this category (Table 1).

TABLE 1

Number of Sets of Corpora Lutea Found in 672 Ovaries From Wild Norway Rats of all Sizes (Body Weight 50–590 gms)

No. of sets of corpora lutea	No. of ovaries	Percent of rats
None (immature)	120	17.9
One	128	18.8
Two	348	51.5
Three	76	11.3

Next was a group of rats having from 4 to 8 corpora lutea in both ovaries, these structures being widely separated by ovarian parenchyma. Any differences there may have been in the size of these corpora lutea as compared with those of older animals were not easily apparent, though actual measurements were not made. Animals showing only one set of corpora lutea (Fig. 1) constituted 18.8 percent of our material (Table 1). It is deduced that these are somewhat older rats that have corpora lutea resulting from initial ovulation.

The majority of animals have two or three sets of corpora lutea. The most frequently encountered number of sets of corpora was two (Fig. 2). These constituted 51.5 percent of the cases in our series. The following distinctions of the cell types in each set of corpora were made under low or high magnification.

A. Some corpora lutea were made up almost exclusively of epithelioid lutein cells. These showed various degrees of luteinization from the relatively newly luteinized cell with homogeneously staining cytoplasm to large luteinized cells having 4–6 times as much cytoplasm and having a lacy appearance to the cytoplasm due to dissolution of the fat granules. The latter cells, hence, had a much lighter appearance than the former ones. Few connective tissue cells are found interspersed between the lutein tissue. It was concluded that these were new corpora.

B. Other corpora lutea had epithelioid lutein cells in various stages of regression. There was always a greater proportion of connective tissue cells to lutein cells in this set of corpora as compared to the new set. Indeed in the more advanced stages of regression the corpora lutea consist predominately of connective tissue cells with only scattered groups of lutein cells in evidence. These were designated as old corpora lutea.

Almost 90 percent of the ovaries examined had none, one or two sets of corpora lutea. Occasionally in addition to the two well-defined sets of corpora there was a set of follicles that had just ovulated. Of these newly ovulated follicles some may show signs of luteinization while others do not. In addition one or two Graafian follicles may be seen with retained ova. In such a case three sets of corpora lutea were noted,

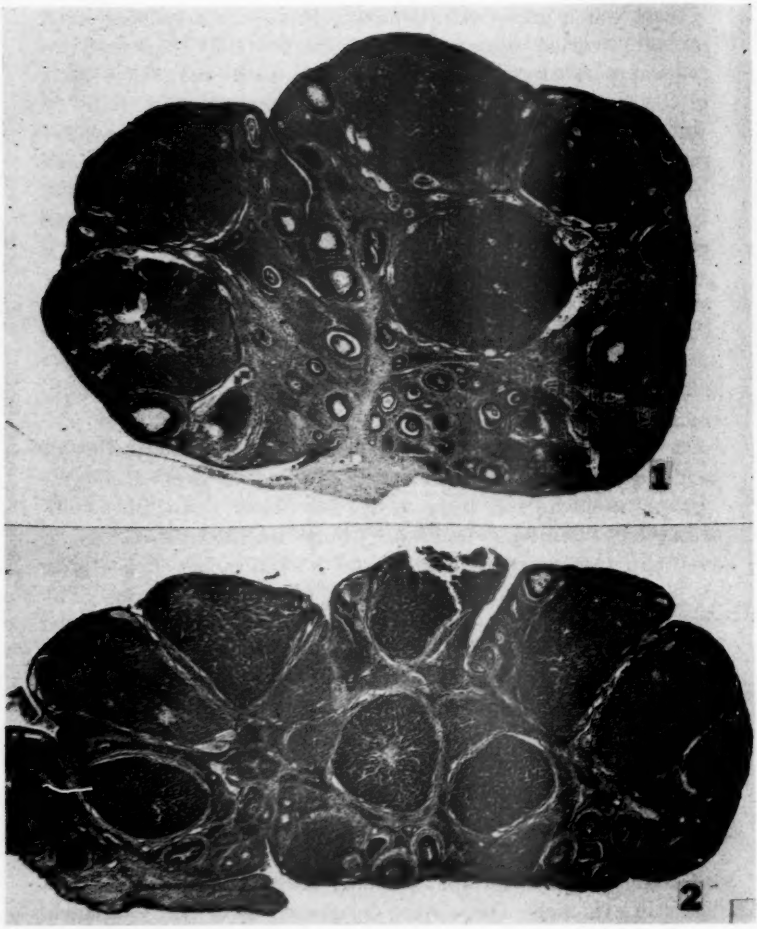


PLATE 1

FIG. 1

Section of an ovary from an adult female whose body weight was 310 gm. One set of five corpora lutea is present here. Mag. X 20.

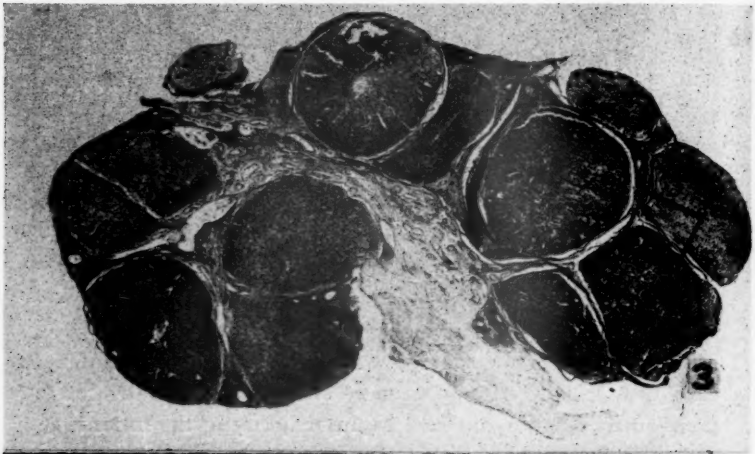
FIG. 2

Section through an ovary showing the presence of 2 sets of corpora lutea —the new set which here appears darker staining and which in this section consists of 5 corpora lutea; and an older set of corpora lutea. Mag. X 20.

the newest being designated as "recent." Finally there was a group of ovaries showing two well-defined sets of corpora lutea and in addition ill-delimited areas in which occasional lutein cells were abundantly surrounded by connective tissue cells. These were corpora lutea involuting to the stages of obscurity, and their presence was noted as "remnants."

In counting corpora lutea and assigning them to their respective sets, it was convenient to first examine the ovary and decide how many such sets were present. When the difference in age between two or more sets of corpora lutea was great enough, as was the case in the majority of the instances, the difference between them was very obvious under low magnification and serial count of the corpora lutea in each set was made. In cases where the difference was not marked, it was necessary to resort to higher magnification to scrutinize the cell types closer, and especially to observe the proportion of connective tissue cells to lutein cells. The older set of corpora lutea would invariably have a greater proportion of connective tissue cells to lutein cells than would the new set (Figs. 3 and 4). With the exception of 9 cases out of 336, decision on the number of sets of corpora present based on morphological differences was not difficult.

After the number of sets of corpora lutea present was decided upon, counts of corpora in each were made serially. We consider serial examination unavoidable if the counts are to be accurate. Often there is great crowding of corpora lutea with consequent distortion of their shape so that only a small portion, or even none of the surface of the corpus, will appear on the surface of the ovary and in these cases serial counts seem indispensable. Without serial examination unexpected features may easily be missed, such as the presence in 64 percent of our material of accessory corpora (Hall, 1950). We searched for possibilities of making corpora counts non-serially, but the more examinations were made the more convinced we were that this tedious method is difficult to circumvent without seriously sacrificing accuracy.



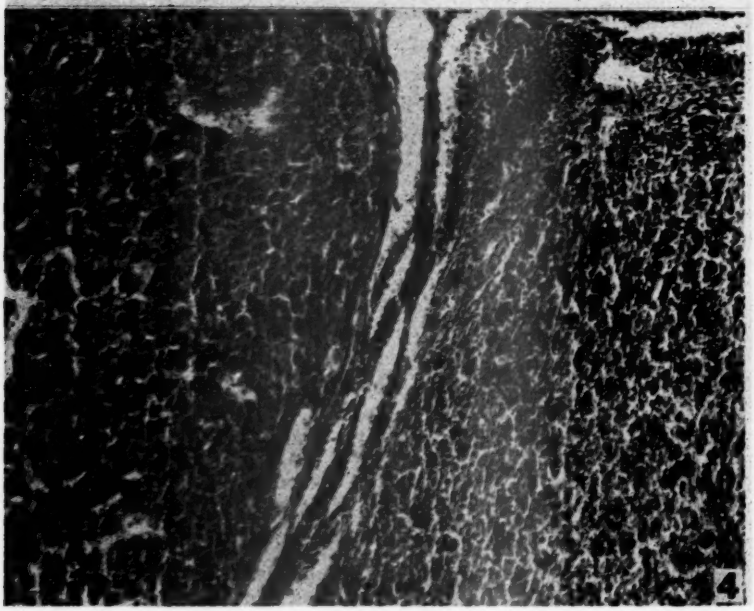


PLATE 2

FIG. 3

Section through an ovary in which two sets of corpora lutea are present, but for accurate determination of this it is necessary to look for the differences under a higher magnification. Mag. X 15.

FIG. 4

Inset of Fig. 3 magnified. A greater proportion of connective tissue cells to lutein cells is present in the old corpus luteum on the right hand, as compared to the proportion of these cells in the new corpus luteum on the left hand. Mag. X 150.

Incidental Observations

Reproductive tracts autopsied when the animals were ovulating (discharged ova being visible in the uterine tube at the time of microscopical study) showed the presence of varying amounts of amorphous staining serous fluid between the ovarian surface and the periovarial capsule. Cellular debris and fluid, in addition to the granulosa cells of the corona radiata around the discharged ovum, were seen in considerable amounts. The discharge of ova from a set of mature Graafian follicles did not seem always to be simultaneous as reported by some workers. Thus some very recent corpora lutea which showed very little or no visible signs of luteinization would be present at the same time as two or three other follicles were rupturing and discharging their ova. Still other Graafian follicles of the same size but with the ova present were apparently about to discharge them even later or not at all. In the latter case they would result in a luteinized Graafian follicle with an entrapped ovum (Hall, 1950).

In the course of microscopic examination sets of corpora lutea which displayed a pronounced "patchy" appearance were seen occasionally. Under low magnification (Fig. 5) this "patchiness" was apparent in all corpora lutea of a set and at most levels of section. Upon examination under a higher magnification (Fig. 6) the "patchy" pattern was found to be made up of lighter areas, irregularly disposed around the periphery of the corpus and consisted of lutein cells with light staining cytoplasm and fat vacuoles. The dark areas were constituted of lutein cells with a homogeneous darkly staining cytoplasm and varying numbers of connective tissue cells. The significance of this observation is not known.

Seen in a small number of rats, approximately 7 percent, were corpora lutea which were very large. They were two to three times the usual size. These were noted as "giant" corpora lutea. Histologically the luteinized cells were as large as those seen in corpora of the usual size, however there were a great number of these cells making the general size of the corpus exaggerated. If very large corpora were present in an ovary all corpora present showed this characteristic. In tabulating

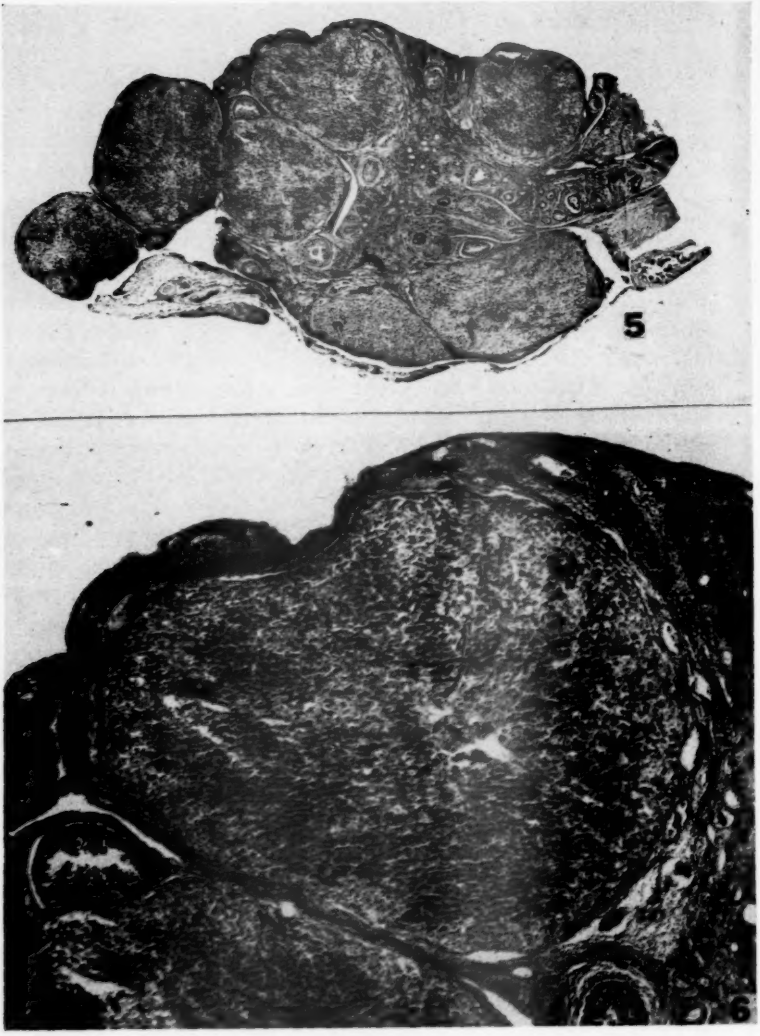


PLATE 3

FIG. 5

Section through an ovary which has one set of corpora lutea present and these show a "patchiness" even under low magnification. Mag. X 15.

FIG. 6

Section showing a corpus luteum with an irregular "patchy" appearance due to groups of lighter staining larger luteinized cells in some areas, while other areas contain groups of cells whose cytoplasm stains darker. Mag. X 60.

most of these cases together with all other information about the rat obtained at autopsy and in subsequent dissection of the reproductive tract, there was no apparent relation between the presence of "giant" corpora lutea and season of the year when these rats were caught, their body weight, the number of embryos recovered from the uterine horns, whether or not they were lactating, pregnant or pregnant and lactating at the time of autopsy. Their significance remains as obscure to us as is their appearance striking.

Evidence of senility in the rat ovary, as described by Wolfe (1943) consisted of presence of wheel cells and cystic follicles which first appear in the year old rats. In our series 17 instances, or three percent of the ovaries examined, showed pathological changes. These consisted of cystic follicles, cystic rete ovarii, one case of ascending inflammation of the uterine tube and ovary, and patchy fatty degeneration of cortical parenchyma (Table 2). In most cases, though these pathological changes were present and in some cases involved as much as half the organ, follicles and several sets of corpora lutea were evident in the normal portion of the ovary, indicating that the organ continued its normal function.

Follicular cysts were seen in older animals, as judged by their body weight and body length, and this may be a sign of senility in this population. If so, only three percent of the animals show signs of old age as estimated by ovarian changes. A note of caution may be added. In extrapolating our knowledge from the laboratory animal maintained under specified conditions of nutrition, cleanliness and care to its wild congener existing in vastly different ecological conditions, and in which for example parasitism is the rule (Andrews and White, 1936), rather than the exception, these differences and other possible ones, should be borne in mind.

Relation of the Number of Corpora Lutea to Embryos

In an attempt to determine the relation between size of female and number of corpora lutea and also frequency of ovulation, several correlations were made. The new corpora lutea counts and the number of sets of corpora lutea were

TABLE 2

Pathologic Changes in Ovarian Structures

Rat No.	Body Weight (Gm)	Body Length (mm)[1]	CORPORA LUTEA "new"[2] left ovary	CORPORA LUTEA "new"[2] right ovary	CORPORA LUTEA "old"[3] left ovary	CORPORA LUTEA "old"[3] right ovary	EMBRYOS IN UTERINE HORNS left	EMBRYOS IN UTERINE HORNS right	CYSTIC Follicles	CYSTIC Rete Ovarii
1	190	175	10	7	0	0	0	0		(1)[4]
2	260	197	5	6	6	9	0	0		(1)
3	264	210	4	6	8	6	0	0		(1)
4	308	214	11	5	9	5	0	0		(1)
5	318	218	6	7	5	6	6	5	(1)	
6	320	218	6	7	6	8	0	0	(1)	
7	330	224	1	5	3	5	0	0	(1)	
8	357	209	4	10	4	7	0	0	(1)	
9	380	223	13	7	6	7	0	0	(1)	
10	386	225	6	6	6	5	5	4		(2)[5]
11	410	211	4	5	9	9	0	0	(1)	
12	422	235	6	8	5	6	6	9	(1)	(1)
13	437	230	8	11	0	0	0	0	(1)	
14	444	212	9	5	8	7	0	0		(1)[6]
15	465	230	2	6	4	2	2	4	(1)	(1)
16	486	253	7	6	0	0	7	6		
17	457	230	4	2	4	4	0	0	(2)	(1)[7]

[1]Head plus body measurement.
[2]"New" refers to the newer set of corpora lutea in that ovary.
[3]"Old" refers to the older set of corpora lutea in that ovary.
[4]One ovary showed these changes.
[5]Both ovaries showed these changes.
[6]Ascending inflammation of ovarian duct and ovary.
[7]Irregular areas of fatty degeneration in cortical parenchyma.

tabulated against the body weights (Table 3) and body lengths (Table 4) to see which groups had the greatest number of corpora lutea and presumably produced most ova at each ovulation, also which groups had most sets of corpora lutea present indicating perhaps more frequent ovulation.

Animals under 100 gms body weight or less than 140 mm in body length did not ovulate, as no corpora lutea were found in ovaries from these groups. Less than 50 percent of the animals in the 100–200 gm body weight group ovulated but from the corpora lutea counts it appears that once they have started to ovulate they discharge almost as many ova as animals that weigh more and are presumably older. Animals over 200 gms body weight (200–550 gms), with one exception, all ovulated; and with one other exception these produced over 6 and up to 25 corpora lutea, with most of them (58 percent) having from 6–15 corpora lutea present in each pair of ovaries. Generally, the greater the body weight and presumably the older the female, the greater number of corpora are found, tending to point to more ova discharged. This assumption should be tempered with the possibility that an unknown proportion of Graafian follicles luteinize without discharging an ovum, and that these may easily be mistaken for corpora lutea in making counts (Hall, 1950). Nor is it known whether the prevalence of such luteinized Graafian follicles is uniformly distributed among the various body weight groups. Greater numbers of sets of corpora lutea also generally go with greater body weight, indicating possibly that there is a greater frequency of ovulation in these groups or that corpora lutea persist longer due to other factors such as greater prevalence of pregnancy with lactation (Table 5).

Analysis of the relation of corpora lutea to embryos shows that in this series there were 1,060 new corpora lutea counted and 915 embryos recovered (Table 6). Thus loss of embryos, or intra-uterine mortality computed on this basis is 15.8 percent. There are, however, several additional considerations to be taken into account. Some of the structures counted as new corpora lutea are undoubtedly luteinized Graafian follicles which did not release an ovum. In cases where remnants of such an ovum are still present in a luteinized structure, it is

TABLE 3

Body-weight Tabulated with Reference to Groups of New Sets of Corpora Lutea and the Number of Sets of Corpora Lutea

Body weights (gms)	RATS No.	% [1]	NUMBER OF NEW CORPORA LUTEA 0 Rats	%	1–5 Rats	%	6–10 Rats	%	11–15 Rats	%	16–20 Rats	%	21–25 Rats	%	NUMBER OF SETS OF CORPORA LUTEA 0 Rats	%	1 Rats	%	2 Rats	%	3 Rats	%
1– 99	15	4.5	15	100											15	100						
100–199	78	23.2	44	56.5	4	5.1	12	15.4	12	15.4	6	7.7			44	56.5	23	29.5	10	12.8	1	1.3
200–299	112	33.4	1	0.9	1	0.9	42	37.5	43	38.4	21	19.2	4	2.8	1	0.9	24	21.4	69	61.5	18	16.1
300–399	87	25.8					26	29.9	49	56.3	8	9.2	4	4.6			10	11.5	61	70.0	16	18.4
400–499	41	12.2					10	24.4	22	53.6	9	22.0					7	17.1	28	68.3	6	14.6
500–599	3	0.9							2	66.7	1	33.3							3	100		

[1]Percentages refer to proportion in weight class having the indicated number of corpora lutea or of sets of corpora lutea.

TABLE 4

Body-length Tabulated with Reference to Groups of New Sets of Corpora Lutea and the Number of Sets of Corpora Lutea

Body length[1] (mm)	RATS		NUMBER OF NEW CORPORA LUTEA												NUMBER OF SETS OF CORPORA LUTEA							
			0		1–5		6–10		11–15		16–20		21–25		0		1		2		3	
	No.	%[2]	Rats	%	Rats	%	Rats	%	Rats	%	Rats	%	Rats	%	Rats	%	Rats	%	Rats	%	Rats	%
100–119	3	0.9	3	100											3	100						
120–139	9	2.9	9	100											9	100						
140–159	30	8.9	23	76.6	2	6.7	3	10.0	2	6.7					23	76.6	5	16.7	2	6.7		
160–179	44	13.1	22	50.0	2	4.5	9	20.4	6	13.7	5	11.4			22	50.0	15	34.1	7	15.9		
180–199	80	23.8	3	3.8	1	1.3	30	37.5	27	33.7	18	22.5	1	1.3	3	3.8	21	26.2	48	60.0	8	10.0
200–219	83	24.7					30	36.2	38	45.8	10	12.0	5	6.0			12	14.5	57	68.6	14	16.9
220–239	74	22.0					18	24.3	41	55.4	12	16.2	3	4.1			10	13.0	53	71.8	11	14.9
240–259	12	3.6					2	16.7	9	75.0	1	8.3							7	58.3	5	41.7
260–279	1	0.3									1	100					1	100				

[1]Head plus body measurement.
[2]Percentage refers to proportion in length class having the indicated number of corpora lutea or sets of corpora lutea.

TABLE 5

Prevalence of Pregnancy with Lactation

Mid-point of body weight (Gms)	No. Rats	PREGNANT No.	PREGNANT %	PREGNANT AND LACTATING No.	PREGNANT AND LACTATING %
50	1	1	100		
150	2	2	100		
250	26	21	80.4	5	19.2
350	45	18	40.0	27	60.0
450	18	9	50.0	9	50.0
550	3	1	33.3	2	66.6
TOTAL	95	52	54.7	43	45.3

TABLE 6

Corpora Lutea Counts Related to Embryo Counts in 95 Pregnant Rats

CLASS	Total Number	Average per Rat	Percentage
Pregnant females (out of 336)	95	...	28.3
New corpora lutea counted in pregnant rats	1,060	11.2	...
Embryos counted	915	9.6	...
Females showing less embryos than corpora lutea	44	...	46.4
Excess of corpora lutea[1]	150	3.4	...
Females showing more embryos than corpora lutea	16	...	16.8
Excess of embryos	35	2.2	...
Females showing excess of corpora lutea in one horn and excess of embryos in the other horn	17	...	17.9
Excess of embryos	37	2.2	...
Excess of corpora lutea	25	1.5	...
Females showing equivalent numbers of corpora lutea and embryos	18	...	18.9
Prevalence of new accessory corpora lutea in ovaries of pregnant rats	56	...	58.9

[1]Some corpora lutea counted as such were large luteinized Graafian follicles which are indistinguishable from corpora lutea, thus this figure of corpora excess is higher than it should be.

possible to avoid counting them as corpora lutea. In instances where there remains no evidence of the trapped ovum, and such we feel is the case in the majority of the instances, there is no way of distinguishing a luteinized structure resulting from a follicle that ovulated from a structure morphologically similar but resulting from a follicle with a trapped ovum. Thus in such cases the corpora lutea count is likely to be higher

than the actual number of ova released, and assuming every ovum released is fertilized (an assumption perhaps not wholly justified) the embryo count will be lower in some instances than expected from corpora lutea counts even if no intra-uterine mortality occurs. Thus the intra-uterine mortality rate would appear higher than it actually is.

On the other hand instances of where greater numbers of embryos were recovered from the uterine horn than the number of new corpora lutea found in the respective ovaries, together with the observation that polyovuly was prevalent in at least 12 percent of the rats in this material led the present authors to conclude that the excess of embryos over new corpora lutea may be accounted for by assuming that some Graafian follicles discharge more than one viable ovum capable of fertilization and giving rise to embryos (Davis and Hall, 1950b).

A case in point may be cited here. Female rat #5667 was recorded as being 426 gms in body weight, lactating and pregnant. When corpora lutea counts were made, one distinct set was present and in addition, remnants of a much older set were noted but not counted because they had regressed beyond the point of accurate count. The two sets of corpora were thus far separated by time and it would not be possible to mistake the separation of the corpora into sets. Thirteen new corpora were counted in both ovaries, 4 in the one and 9 in the other. However, 23 embryos, 9 mm in length, were recovered at the time the uterine horns were dissected with 5 embryos being found in one horn and 18 in the other. This was an unusual case, admittedly, and for that reason it was checked for possible error or explanation but none could be found. Here no question could arise as to the number of corpora present nor as to the number of embryos counted, both counts having been made and checked by one of the authors and a technician separately and together. This example is undoubtedly, and perhaps fortuitously, exaggerated for us by Nature, but also it seems to indicate the potentialities that apparently exist.

Thus attempts to estimate accurately intra-uterine mortality by the approach discussed here, and used by some authors

(Perry, 1945; Brambell, 1944), studying wild populations would be clouded by these two factors which tend in opposite directions—one tending to make the intra-uterine mortality appear higher than actual while the other would make the intra-uterine mortality appear smaller than it may be.

From the point of view of the physiology of reproduction it may be more desirable to equate the numbers of embryos recovered from one horn to the number of corpora lutea in the ovary on that side. It seems improbable that transmigration of ova from one ovary across the abdominal cavity to the uterine horn on the other side occurs in the rat as it apparently does in such species as the rabbit (Brambell, 1944). A well developed periovarial capsule which encloses the ovary is plainly visible in our sections and has been previously described. The fimbria are equally plainly enclosed within the capsule in our material. Until rather recently it was thought this periovarial capsule was completely closed at all times. Alden (1942) has shown, however, that in the laboratory rat it is not completely closed all times but is considered to be so at the time of ovulation. When the new corpora lutea of each ovary were equated to the embryos found in the horn on the same side, there were cases with fewer embryos than expected in the one horn and more embryos than expected in the other horn (Table 6).

If transmigration of ova across the abdominal cavity is ruled out, also migration of blastocysts from one uterine horn to the other because of the presence of a separation between the two horns in the region of their fusion and the presence of two ora uteri, than in the case of excess of corpora lutea several possibilities exist. Some luteinized Graafian follicles which did not release an ovum were included in the corpora lutea count; or the released ova did not become fertilized; or if fertilized, the ova failed to form a normal blastocyst and to implant. In examining discharged ova and blastocysts in uterine tubes in some of our material we did notice that the staining capacity of a group of ova or blastocysts was not always uniform. But whether this indicated that some were improperly developed and would fail to implant we were not able to conclude from this study. In the case where more

embryos were recovered than corpora lutea counted again several explanations may be suggested. Some ova gave rise to more than one individual, that is, twinning occurred; or some follicles gave rise to more than one ovum, that is, polyovuly occurred, and these ova were capable of fertilization and implantation.

Among the pregnant female group as a whole, making no distinction between those females that may be pregnant for the first time and those that were already old enough to have had more than one litter, there was coexistence of lactation and pregnance in 45.3 percent of the cases (Table 5). If only the more mature females from 300 gm body weight (this weight also includes the weight of embryos in pregnant females therefore the actual weight of the mother is less than this) and up are considered, 59 percent of these are found to show lactation existing with pregnancy. Lactation during pregnancy is known to influence the gestation period. When pregnancy results from the post-partum estrus in the rat, the ova are fertilized and continue to divide in the usual manner to form blastocysts. However, the blastocysts lie free in the uterus without implanting for some time. The delay in implantation may be from two to fifteen days, varying roughly with the number of young that the mother is suckling (Hamlett, 1935).

Summary

Ovaries from 336 female wild Norway rats were submitted to serial histological examination for counts of the sets of corpora lutea present and the number of corpora in each set. Uterine horns of pregnant female rats were also dissected and embryos recovered for count and measurement.

The number of sets of corpora lutea found in the wild Norway rat ovaries was smaller than the numbers reported for the laboratory Norway rat. Thus of the 672 ovaries studied: 17.9% had no corpora lutea present (immature rats), 18.8% showed one set of corpora, while 11.3% had three sets of corpora lutea present at the same time.

In the whole group of pregnant rats, with a body weight range from 50–600 gms, 45.3% showed the coexistence of pregnancy with lactation. This included those rats that were pregnant for the first time and therefore were not exposed

to the possibility of a pregnancy resulting from fertilization at the time of post-partum estrus. If females of 300 gm body weight and more are considered, 59% showed the presence of pregnancy with lactation.

Lactation is known to prolong the gestation period, when the two conditions coexist. It is considered that this would prolong the time lapses between periods of estrus and therefore the formation of new sets of corpora lutea, and thus result in fewer sets of corpora lutea as encountered in this material.

The degree of accuracy of the correlation between the number of corpora lutea counted in the ovaries and the number of embryos recovered from the respective uterine horns is discussed.

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PATHOLOGY OF THE NEUROHYPOPHYSIS WITH SPECIAL REFERENCE TO DIABETES INSIPIDUS

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The only clinical syndrome unequivocally related to disease of the neurohypophysis is diabetes insipidus, which was defined by Johann Peter Frank in 1794 (Futcher, 1931) as "a long-continued abnormally increased secretion of non-saccharine urine which is not caused by kidney disease." The essential lesion is thought to be an interruption somewhere along the nervous pathway extending from the supraoptic nucleus in the anterior hypothalamus to the neurohypophysis, which is called the hypothalamico-hypophyseal tract (Fisher, Ingram and Ranson, 1938), and the essential physiological defect is failure of the kidney tubules to reabsorb water (Peters, 1944), so that the patient excretes urine that is always very dilute and usually copious in amount.

Clinical Consideration of Diabetes Insipidus

Since any lesion affecting the neurohypophysis or the anterior hypothalamus is capable of causing DI (diabetes insipidus), specific etiology can be better indicated than defined, by citation of patient surveys published by Fink (1928) and by Jones (1944) (Table 1). Fink made a clinico-pathologic sur-

TABLE 1

Series of Fink (1928); 107 autopsied cases taken from literature:

Cause	No. cases	Percent
Tumor—base of brain or posterior	68	63%
Lues—meningitis or gumma	14	13%
Tuberculosis—meningitis or tuberculoma	5	
Non-luetic inflammation	9	
Traumatic	11	

vey of 107 autopsied cases collected from the world literature. Many of these cases were taken from very early literature with

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inadequate pathological data. Many of the tumors listed were of little localizing value since they extensively involved the base of the brain, but 15 of the 68 tumor cases had only a solitary nodule in the hypophysis or infundibulum, primary or metastatic. Several were reported to have had a normal hypophysis, but all had neighborhood lesions which conceivably might involve or compress the hypothalamus or pituitary. The

TABLE 2

Series of Jones (1944); cases seen at University of Michigan Hospital between 1920 and 1944 with daily water exchange of at least 6 liters intake/liters output and specific gravity never exceeding 1.008.

Cause	No.	Cases
Tumors	17	
*Pituitary		11
Hypothalamus		2
Xanthomotasis		4
Infectious	10	
†Chronic encephalitis		7
‡Lues		3
Vascular	3	
§Pituitary infarction (?)		1
‖CVA		1
Subarachnoid hemmorhage	1	
Trauma		3
¶Undetermined		9

*1 case followed surgical transection of the stalk at operation for excision of tumor and subsided after after about six month (not an uncommon phenomenon with post-traumatic DI.

†1 had narcolepsy.

‡Only 2 had evidence of CNS lues.

§Presumptive; 2 days post-abortion, septic.

‖Clinically, a sudden hemiplegia.

¶1 was associated with an acute delirium, cause unknown; 1 was associated with menopause, abnormal EEG, S.F. protein of 70 mg.: 1 was associated with psychosis; 1 occurred in a patient with apparent hysteria, but she had abnormal concentration test.

series of Jones includes all cases seen at the University of Michigan Hospital between 1920 and 1944 which fitted the criteria of daily water exchange of at least 6 liters intake/4 liters output (urine), and urinary specific gravity never exceeding 1.008. Of these only two had adequate autopsies, both found to have tumors. Only six of the entire series had urine concentration tests.

The cases seen at John Sealy Hospital between 1920 and 1950 are tabulated for comparison (Table 3), although the series

TABLE 3

Cases seen at John Sealy Hospital from 1920 to 1950:

Total cases diagnosed (from Record Library Kardex)	17
Charts not found	5
Misdiagnosed (from data on charts)	4
Total cases with presumed correct diagnosis	12
*Tumor	2
Trauma (skull fracture)	1
Xanthomatosis	2
†Post-encephalitic	1
Associated with mental deficiency	1
Cause unknown	4

*One of these was presumptively diagnosed on basis of third ventricle displacement on X-ray; neither case came to surgery.
†Patient developed obesity concomitantly with DI.

is too small to be of any significance. No patient came to autopsy. No patient had a family history of DI. Only five of the 12 adequately (?) studied cases met the criteria of Jones as to fluid exchange and urine specific gravity.

Tumors causing DI have numbered every type of growth, including Hodgkin's, Boeck's sarcoid, leukemia, teratoma, sarcoma, craniopharyngeal cyst, carcinoma of the posterior lobe, and metastatic tumors of all varieties (Warkany and Mitchell, 1939). Pinealoma is often associated with DI, especially the "ectopic" variety (Horrax and Bailey, 1925; Horrax, 1947; Horrax and Wyatt, 1947). Chaismal gliomata are accompanied by DI in 20% of cases. Sometimes the causal mechanism appears to be increased pressure from obstruction of the ventricular system by tumor with resultant internal hydrocephalus. Internal hydrocephalus with associated DI due to actinomycosis has been reported. Oppenheim (1895) found DI in 12 of 36 patients with meningitis. Tuberculosis is a relatively uncommon cause today. Encephalitis caused by measles, mumps, and pertussis has been followed by DI, and one case of encephalitic origin demonstrated graphically the prevalent etiological theory at post mortem, with the finding of bilateral degeneration of the supraoptic nuclei and of the pituitary (McGavack *et al.*, 1940) Cases have followed extension of purulent sphenoidal

sinusitis (Ball & Thackeray, 1948, Yaskin *et al.*, 1942). Vascular causes appear to be uncommon, being either hemorrhage (Jones 1944) or aneurysm; in the latter case, hemorrhage from rupture of the aneurysm may result in improvement of symptoms (Loewenberg and Sloane, 1940). Spain and Geohagan (1946) reported two cases with acute postpartum necrosis of the pituitary. Both cases remained in non-hemorrhagic shock following delivery until death, and a small amount of histologically normal hypophyseal tissue remained in each case. The kidneys of one patient were normal, the other showing slight tubular degeneration. Of the 95 cases of histologically proved Simmonds Disease reported by Sheean and Summers in 1949, none of those with postpartum necrosis had DI, but DI had been present in one case of massive tuberculous caseation, one or two (?) with massive granulomatous necrosis of uncertain etiology, and three with granuloma of the hypothalamus. DI symptoms ranged from a few weeks to 2 years before death. In addition, three cases with healed gummatous necrosis had had transient DI 10 to 24 years previously. Occasionally DI is associated with obesity, sexual precocity, and sleep disturbance (narcolepsy) when the lesion extensively involves the hypothalamus.

Fink felt that hereditary DI was rare or even coincidental, apparently on the basis of autopsied cases, and Blotner (1942) obtained a family history of the disease from only 1 of 70 patients, but there are a number of reports of such patients (see Warkany and Mitchell, 1939), who are usually unaffected otherwise. The most remarkable is that of the Weils, father and son; Adolph in 1884 reported a family of 91 persons in four generations, 23 of whom had DI. His son, Alfred, continued these studies over a fifth generation of this family and found among 200 members a total of 35 in whom DI had developed. Hereditary transmission from a parent to an offspring was direct in all but one instance. Of all the children of affected parents, more than half the boys and one third of the girls suffered from the disease. Polyuria and polydipsia, while probably present at birth in these cases, were noted definitely during the second year. The height of the disorder was reached between the ages of 20 and 30 years, and symptoms slowly

improved after the age of 50, disappearing entirely a few days before death. During fever, the specific gravity of the urine might reach 1.018. Some of the affected people lived to such ages as 83, 87, and 94. The sexual functions were normal and the fecundity of the family was above average. No difficulties were encountered by the affected women during pregnancy or delivery.

The overall incidence of DI is about 0.01% of hospital patients (Fitz, 1914; Fink, 1929). On the basis of comparison of the autopsied series with the clinical series as noted above, and with the considerable number of reports in the literature of "idiopathic" cases, it seems likely that the actual incidence may be quite different, and particularly that the indicated number of idiopathic cases of DI may be falsely low. Age incidence will be indicated by this random tabulation of some of the publications encountered during the preparation of this review:

Age at Diagnosis	Number of Cases
0–10	22
11–20	20
21–30	17
31–50	20
over 50	5

The idiopathic variety is most commonly reported in young people or children, and in those cases with a history of symptoms since birth or soon after, many have concomitant congenital anomalies or hereditary disease (DI) (Warkany and Mitchell, 1939).

The only constant symptoms of DI are polydipsia and polyuria, which are mutually compensating in respect to maintaining water balance in most cases. After trauma, the onset may be sudden; traumatic cases are peculiar in that not only is the onset well-defined, but the first symptom may be an overpowering thirst, preceding polyuria by several hours to several days. Kourilsky (1950) describes such a case in whom relief of polydipsia followed immediately on tapping a subarachnoid cyst impinging on the hypothalamus, although polyuria was not relieved until many hours later. Warkany and Mitchell (1939) wisely suggest however that often an increased volume of urine secretion may precede micturition by several hours, particularly in a person who has just suffered a severe trauma, who has not

been completely conscious, and who therefore may not void. The primacy of thirst over polyuria received some support from Pasqualini and Avogadro (1942) who reported that pitressin given without HOH relieved thirst in 2 patients with DI.

Experimental data tends to support the concept that the thirst of DI is consequent to water depletion from polyuria. Richter (1935) was able to abolish the polydipsia of rats with experimental DI by ligating the ureters. Ranson and associates (1938) observed that after placement of lesions in the vicinity of the hypothalamus and hypophysis such as to cause DI, polyuria invariably preceded polydipsia, sometimes by as much as three days. Thirst in their animals could be relieved by feeding water per stomach tube. It is possible to relieve thirst in human DI by forcing water; this overhydration also shows other pitressin-like effects—increase in plasma and extra-cellular fluid volume, decrease in hematocrit and in serum sodium and protein and in urine output (Holmes and Gregerson, 1948). No "thirst" center has been demonstrated in the vicinity of the neurophypophysis, and despite rather clearcut direct evidence in a very few human cases to the contrary, the above mentioned indirect evidence supports the concept that the thirst of DI is caused by the same type and degree of dehydration as normal thirst. In this connection, a very suggestive phenomenon was reported by Kuntzmann (1933) who himself drank 10 liters of water daily for several days, then suffered torturing thirst for some days afterward; this sort of "habitual" or "conditioned" drinking might well explain some cases which fail to respond to hormonal treatment.

Frequency of micturition accompanies the abundant urination, which in occasional patients is greater than 30 liters. Some patients are not satisfied with ingestion of water alone, and as consequence, physicians have had opportunity to observe an unusual alcohol tolerance in DI, as yet unexplained (Warkany and Mitchell, 1939); whether this phenomenon accompanies all cases of DI is unknown. Restriction of fluid intake leads to collapse in both animals and man, as well as to a decrease in chloride and creatinine clearance and to an increased "water-binding power of protein" (Greiner and Podjradszky, 1947). The body temperature is said to be slightly

subnormal, due to heat loss connected with the enormous water exchange (Rowntree, 1921, 1924). The urinary bladder and ureters are sometime found to be dilated when the disease has been present for a long time. The heart is normal. Pathology in the kidneys, by definition, is unnecessary, and does not often occur.

When DI is secondary to another disease, the symptomatology becomes exceedingly diverse, although predominantly neurological in character. It should be kept in mind that DI is often associated with tumor at the base of the brain, and the symptoms of DI should require the exclusion of a lesion in this vicinity.

If the patient is in good state of nutrition and hydration, laboratory data are essentially normal except for the finding of persistently low urinary specific gravity, with a low concentration of solids in the presence of a normal total output of solids. The specific gravity remains low even after fluid restriction. Kidney function, as measured by dye excretion or urea clearance has been reported to be impaired (deLawter, 1949) and improved (Hachen, 1923; Green and January, 1940) after hormonal replacement treatment. DI was at one time divided into so-called "hyperchloremic-hypochloruric" and "hypochloremic-hyperchloruric" types (Veil, 1918), and it was thought that the former was produced by a diencephalic lesion, the latter by a 4th ventricle lesion. This distinction does not appear to be valid clinically. Blotner (1941) states that with normal intake, chloride balance is normal. The serum proteins are usually normal, but it has been claimed that their colloid-osmotic pressure is increased (Barath and Weiner, 1935). Basal metabolism rate is said to be less than — 15 in a symptomatic patient (Warkany and Mitchell, 1939).

The diagnosis of DI is made on the basis of excessive water exchange and low urinary specific gravity, after other possible causes have been ruled out. Diabetes mellitus is rarely associated with DI, being ordinarily detected only when polyuria persists after adequate control of the hyperglycemia and glycosuria. The possibility of hypophyseal causation of the diabetes mellitus in these cases has not been adequately investigated, although in the case of Talbott *et al.* 1940, the inference

of a hyperplastic and hyperfunctioning anterior pituitary encroaching upon the supraoptico-hypothalamic pathway was supported by roentgenography of the sella turcica; in this patient diabetes mellitus preceded DI.

Polyuria often occurs in advanced pellagra, disappearing with adequate treatment (Rassulev, 1932). Several cases of an apparently congenital syndrome resembling DI have been reported in children in which a fever was a prominent symptom. In spite of disturbed electrolyte balance and certain other atypical features, these probably are actual cases of DI distorted by a superimposed or causative illness (Waring *et al.*, 1945; Dancis *et al.*, 1946). Williams (1946) described a group of patients with nephrogenic DI occurring only in males of one family and transmitted by the females. Pitressin and pituitrin were ineffective, but he was able to demonstrate that these agents were not inactivated by the serum of one patient after a period of an hour. Primary polydipsia in children, which presumably corresponds practically to psychogenic or hysterical polydipsia-polyuria in adults, often is difficult to separate, but an adequately performed concentration test after fluid restriction, in the absence of renal disease, is ordinarily diagnostic. Various renal affections may cause polyuria and/or low specific gravity; the difficulty which may sometimes arise in making a diagnosis may be illustrated by the case of E. J., a 30 year old Negro female recently under the care of Dr. E. D. Futch in John Sealy Hospital. She was admitted to the ward in heart failure, and was noticed to be polyuric as her cardiac decompensation cleared up. Review of the chart revealed that even while in failure, she had passed large volumes of urine. Her urinary specific gravity had never been greater than 1.010, usually standing at 1.000 to 1.002, and renal disease was thought to be present, in part on the basis of dye excretion of only 30% in two hours. However a test dose of pitressin increased the specific gravity of the urine to 1.022 and 1.018 on two occasions. Further treatment reduced urine output to 1500–2000 ml. per day, but the dye excretion test did not improve. This calls attention to the value of pitressin as a diagnostic test for DI.

Another proposed test relies on the response of the patient to infused sodium chloride (Carter and Robbins, 1948). After a period of fluid restriction, the patient is hydrated with 20 ml. water per kg. body weight, then infused with a 2.5% solution of NaCl for one hour. The insipidly diabetic patients show a slight rise to a slight fall in urine output during this period, while normal persons and patients with polyuria from diabetes mellitus, hysteria, and calcareous renal disease show a pronounced fall in urine output after a few minutes. Unfortunately no patients with chronic glomerulonephritis were included in this series.

Treatment of DI presupposes treatment of the causative disease if such is feasible. Considerable success has been reported in some quarters (Rowntree 1921, 1924) in relief of symptoms of disease secondary to syphilis by anti-luetic therapy. Cures or improvement have also been reported after surgical removal of tumor in the vicinity of the hypophysis, by irradiation of the hypophysis in xanthomatosis, and in one case by irradiation of this region when the cause was thought to be an extension of the middle ear infection (Weinstein and Spingarn, 1940). The double-edged character of the surgical blade is indicated by the previously cited case of Jones (1944), in whom DI followed cutting the pituitary stalk in the course of excision of a tumor.

The only specific treatment for DI is hormonal replacement, namely posterior pituitary extract, or the pitressin fraction thereof, which contains the anti-diuretic principle and from which it has not yet been separated for commercial exploitation, although Heller did perform a separation as early as 1940. It would appear that powdered posterior pituitary extract and pitressin tannate offer the best forms of treatment, the former because of its economy, and the latter because of its long action. In addition, both seem to be freer of the distressing side-effects of aqueous pitressin and pituitrin, *i.e.*, palpitation, pains in the chest and abdomen, dizziness, headache, flushing, menorrhagia (Plass, 1948, Rutledge and Rynearson 1939), etc. The powder is used like snuff, intra-nasally; it may occasionally cause rhinitis when frequently used (Hart and Breitman 1941). deLawter's (1949) patient was unable to tolerate nasal use of the material,

but got excellent results from intra-vaginal application. Powder must be applied three to four times daily for complete control. Aqueous pituitrin has been used with an atomizer for nasal spray (Blumgart, 1922). Pitressin tannate in oil controls symptoms from 24 to 36 hours following intramuscular injection. Lederer (1949) recently reported the addition of a polyvinylpyrrolidone to pituitrin solution. This compound prolonged the antidiuretic action of a single dose to two to four day, permitted the use of smaller doses, and eliminated most side effects; the pituitrin molecule was presumably adsorbed with delay of absorption of the injected material.

An allergic type reaction to aqueous pitressin has been reported (Plass, 1948), consisting of giant urticaria, erythema over the site of injection malaise, aching, stiffness of the hands, feet, and lips, comiting, and tetany with carpo-pedal spasm on one occasion. An intra-cutaneous test in this patient produced a 4–5 cm. wheal. Benadryl permitted continuation of treatment with pitressin.

A certain number of cases will respond to only one, and some to none, of the available preparations. There is sometimes very poor correlation between the amount of fluid exchange and the amount of drug required for control. In using pituitrin solution, caution is advised in the presence of coronary disease, hypertension, epilepsy, and pregnancy (Sollmann, 1948); Soule's (1937) patient began in the 37th week of gestation to have "irregular painful contractions" following insufflation intervals of powder.

Restriction of dietary sodium chloride will diminish the diuresis of DI, and as such is sometimes a valuable adjunct to hormonal treatment. Addition of sodium chloride to the diet of the DI patient will often greatly increase his diuresis. Protein restriction has also been recommended (Allen and Sherrill, 1923) but is probably of not much value unless carried to a radical extreme.

Surgical treatment other than for removal of tumor invading or compressing the hypophyseal region is of interest more for physiological considerations than for practical application. Strauss in 1920 reported the case of an adolescent girl whose

symptoms disappeared following the onset of spontaneous myxedema. Findley and Heinbecker (1937) reported a case in whom the disease was presumably of luetic origin but failed to respond to antiluetic treatment. On the strength of the animal experiments of Mahoney and Sheean (1935), which will be described later, thyroidectomy was performed, but there was no greater diminution of diuresis than had previously been induced by a low-salt diet alone. However, in two patients whose DI had followed encephalitis, and who had moderately elevated basal metabolic rates, total thyroidectomy decreased water exchange to less than 3500 ml./day. Administration of desiccated thyroid re-established diuresis which was not so severe nor as persistent as before surgery. The rise in fluid exchange after thyroid occurred before the BMR rose again, and there was no increase of water exchange associated with elevated BMR from dinitrophenol treatment.

Hirsch (1936) transplanted human hypophysis into the rectus muscle of two patients with post-traumatic DI and reported a decrease in urine volumes, and urine concentration and dilution reactions similar to those of normal persons.

Adequate control or improvement of symptoms has been attributed to lumbar puncture, encephalography, ergotine, parenteral injection of milk, short-wave irradiation of the pituitary and diencephalon, aspirin, amidopyrine, and antipyrine, intermedin, antuitrin, and amniotin (Blotner, 1937; Kahn, 1933; Schube, 1933; Sulzberger, 1933; Turner, 1935; Warkany and Mitchell, 1939; Stübinger and Wolf, 1949). The patient who receives no benefit from the usual hormonal therapy must be carefully evaluated for additional treatment. The amidopyrine group of drugs and intermedin, if available, would seem to be first choices. The newer anti-thyroid drugs offer a convenient medical approach to those patients in whom the thyroid gland may be a factor.

Clinical Considerations Other Than Diabetes Insipidus

No disease or syndrome attributed to a hyper or eu-secretory state of the posterior lobe has been described. Assays of antidiuretic activity in blood and urine have led to the conclusion that the functional state of the neurohypophysis may vary

considerably in health and disease, and particularly, that in certain diseases a state of hyperfunction may exist. Many of these bioassays must be interpreted with caution, in that the antidiuretic activity may represent merely "the algebraic sum of antidiuretic and diuretic substances in the blood" (Lloyd and Lobotsky, 1950), and nobody has yet been in a position to assert positively that such an antidiuretic substance was identical with the hormone of the posterior lobe.

In one group of patients, Robinson and Farr (1940) found a general correlation between antidiuretic activity of the urine and amount of edema. Two patients with normal serum proteins became edema free without change in protein level, and simultaneously the urine lost its antidiuretic activity. The same was observed in four cases of acute nephritis after recovery. They also gave 30 units of pitressin to four patients for 7 days and observed that the antidiuretic effect lasted only about one day, and that the urine passed by three of the patients possessed a diuretic effect by the seventh day. The disappearance of antidiuretic activity paralleled the disappearance of edema which had appeared with initial medication. Ralli *et al.* (1940) confirmed and extended this data with reference to cirrhosis and believed from extraction characteristics that the antidiuretic material was of neuro-hypophyseal origin. Lloyd and Lobotsky (1950) found pronounced antidiuretic activity in the serum of patients with Laennec's cirrhosis, which diminished with water loss. They suggested that normal destruction of the hormone had not occurred due to liver damage (Eversole *et al.*, 1949). In normal patients with premenstrual edema, they found high serum antidiuretic activity immediately premenstrually which fell when the normal diuresis ensued with menstruation. Teel and Reid (1939) recovered from the urine of eclamptic patients large amounts of antidiuretic substance, and observed further that eclamptics were much more sensitive than other pregnant women to small subcutaneous doses of pituitrin. Suppression of anterior pituitary diuretic activity by estrogens has been blamed for edema associated with menses and pregnancy (Schapiro, 1938), although others have attributed this to the salt-retaining property of estrogens. Other studies of the antidiuretic activity of urine and blood serum

from human pregnancy with or without eclampsia are somewhat at odds with one another, but it appears that there is during normal late pregnancy a supernormal amount of antidiuretic substance in serum and urine (Blazso and Dubransky 1941; Krieger and Kilvington, 1946).

One report with pressor implications concerned a group of hypersensitive patients whose blood serum contained antidiuretic activity which disappeared in most cases after irradiation of the pituitary gland; this was followed by alleged clinical improvement in about 50% of cases. Of incidental interest is that none of these patients showed signs of pituitary insufficiency after treatment.

It has been shown (Brun *et al.*, 1946) that the oliguria and chloruresis following syncope induced in humans by water loading in the passive erect position is in many ways similar to the effects of exogenous antidiuretic hormone. Subsequently Taylor and Noble (1950) found antidiuretic activity in the urine of patients subjected to dehydration, patients who fainted spontaneously or in the course of venesection, in patients after electroconvulsive therapy and after IV injection of posterior lobe hormone. They found no activity in the urine of normal individuals, in patients with venesection who did not faint, in patients in whom blackout was induced by experimental acceleration, nor in patients with pre-eclampsia.

Pathophysiology of the Neurohypophysis

The experimental study of the neurohypophysis has been largely concerned with its effects on water balance, and as such is usually dated to Claude Bernard (1849), who produced polyuria and glycosuria separately by piqûre punctures of adjacent areas on the floor of the fourth ventricle, and to Eckhard (1869, 1872) who induced polyuria by punctures in the pons and cerebellum, although the latter is obviously only incidentally related. The neurohypophysis was first definitely related to DI in a clinico-pathologic study by Erdheim in 1904. From observations on anesthetized animals it was reported that posterior pituitary extract was possessed of a diuretic effect (Magnus and Schafer, 1901; Schafer and Herring, 1906), and this work was not reexamined critically until the discovery of

Van den Velden and Farini in 1931, working independently, of the antidiuretic action of this substance in patients with DI. Before this however, Crowe *et al.*, (1910) found that if anterior pituitary tissue was implanted in hypophysectomised animals, DI ensued, but disappeared after removal of the implant. The significance of this was apparently not fully appreciated until 1918 when von Hann postulated that deficiency of the posterior pituitary caused DI only when functional anterior lobe tissue was present. Direct evidence of the physiological action of the neurohypophysis was recently supplied by Harris (1947) who produced typical pituitrin effects on blood pressure, the uterus and urine flow by electrical stimulation of the neurohypophysis in conscious rabbits.

The hypothalamus was implicated by Camus and Roussy in a series of experiments (1913, 1920) in which they were unable to produce DI by hypophysectomy (*cf.* Heinbecker below), but did cause DI by injection of the hypothalamus, especially near the tuber cinereum. Pituitrin appeared to have no effect on their hypothalamic diabetes; this must have been due to the type of extract available to them. Hypothalamic diabetes has been confirmed by others (Bailey and Bremer, 1921; Bourquin, Richter, 1930). Using as criteria the rate of disposal of a water load and the water/food intake ration, Stevenson (1949) recently reported water balance changes in rats subjected to lesions bilaterally of the ventrolateral or ventromedial hypothalamic nuclei, which he interpreted as representing a hyperfunctioning posterior lobe. In the light of present knowledge, this finding is difficult to interpret, although implications are obvious.

Still a third line of thinking was suggested by Cajal's demonstration in 1911 of nerve fibers in the posterior lobe. Pines (1925), 1926) and Greving (1925, 1926) demonstrated the presence of a bundle of nerve fibers passing from the anterior hypothalamus, mostly originating in the supra-optic nucleus, to the posterior lobe, and the following year Zadek (1927) suggested that DI might result from a lesion anywhere along this supraoptic-hypophyseal nerve tract. He called attention to the frequent association of DI with dystrophia adiposogenitalis,

citing Cushing and Maddox (1925) who produced both by clipping the stalk.

This latter conception was the direct antecedent of the classical work of Ranson *et al.*, summarized in their book in 1938. They performed an ingenious series of experiments using the Horsley-Clark apparatus to produce bilateral lesions in the anterior hypothalamus. Histologically those lesions which were followed by DI were shown to interrupt the supra-optico-hypophyseal tract. In addition, there was a retrograde atrophy of the supraoptic nucleus and great thinning of the median eminence, the infundibular stem, and the neurohypophysis, with hypercellularity of an apparently new or altered cell type. They were able to establish a correlation between the severity of DI and the extent of lesions, and also the changes in the neurophypophysis and the supraoptic nucleus could be correlated with the degree of interruption of the supraoptico-hypophyseal tracts. They estimated that more than half the tracts must be destroyed bilaterally before any water balance disturbance occurs. DI could also be induced by section of the infundibular stalk or by neurohypophysectomy, provided in both cases with the median eminence and the infundibulum were either destroyed or damaged. The anterior lobe was undamaged in most cases, and whenever polyuria occurred, normal anterior lobe tissue could be demonstrated. This was supported by normal appearance of the gonads, adrenals, and thyroid. In these animals, the intermediate lobe was normal. Some writers, especially Richter (1938), have felt that maximal DI could be induced by removal of the intermediate lobe alone, and as was noted before, intermedin, the extract of this lobe, has been reported to the efficacious against DI (Sulzberger, 1933). Lesions of the posterior hypothalamus with interruption of the tubero-hypophyseal tract caused no increase in urine output or changes in the posterior lobe. Lesions of the pineal body cause no polyuria (Martin and Davis, 1921). The polyuria of these cats responded to pitressin. Several of the glands removed from autopsied diabetic animals were assayed and found to have very low values for antidiuretic, pressor and oxytocic activity.

The course of the experimental disease consisted of an initial transient polyuria with a peak on the second postoperative day, the fluid exchange declining to normal by the sixth day and remaining at this level until the 11th or 12th day, at which time urine output rose suddenly, followed one to three days later by an increase in fluid intake. This fluid exchange reached a stable maximum in three or four days, being termed then the "permanent phase of DI." Gersh and Brooks (1941) propose that the "normal interphase" between the temporary and permanent phases may be correlated with a "compensatory hypertrophy" or remaining undenervated gland cells which then either revert to normal or undergo "exhaustion atrophy," depending on the amount of nerve tissue destroyed.

Heinbecker *et al.* (1947) confirmed and clarified the necessity of "total" posterior hypophysectomy, (*i.e.*, including the median eminence and infundibulum) for inducing permanent polyuria. However they conclude on the basis of careful studies that (a) the presence of functioning pars distalis tissue is not essential to a marked DI lasting many months, but is essential to permanent maximum maintenance of the diabetic state through its trophic effects on the thyroid and adrenal cortex, and (b) moderate DI may exist in the presence of pars distalis when the residuum of functional neuro-hypophysis is sufficient to prevent polyuria in the absence of the pars distalis. (5% N-H prevents DI.)

The controversy regarding the nature of the diuretic activity of the anterior lobe is not fully resolved. Richter (1934) thought that its effect was mediated through the known hormones, although some recent experiments of Pickford and Ritchie (1945) suggest the possibility of a diuretic effect over and above the trophic action on adrenals and thyroid. Mahoney and Sheean (1935) clearly established the role of the thyroid by thyroidectomizing dogs who had previously been made polyuric by clipping the pituitary stalk. The polyuria was thereby abolished, but could be re-established at will by giving desiccated thyroid (*cf.* Blotner and Cutler, 1941). Radcliffe (1943) confirmed this work in part, noting also a diminished Cl— excretion associated with polyuria in

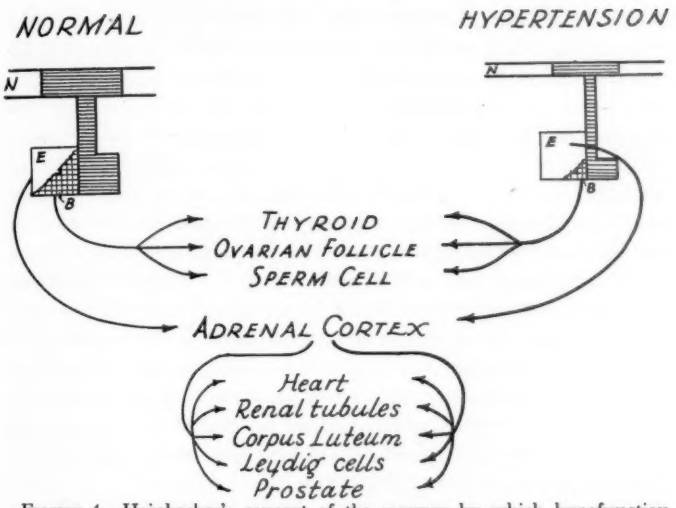

FIGURE 1. Heinbecker's concept of the manner by which hypofunction of the posterior lobe may result in relative hyperadrenocorticism. (Courtesy Dr. Thomas Findley, 1949).

DI cats after thyroid feeding. In normal animals the Cl— effect was present but no polyuria occurred. Some investigators have asserted that anterior lobe extract has no effect on DI after thyroidectomy (Findley, 1937). In the totally hypophysectomized dog, the administration of desoxycorticosterone acetate (DOCA) and desiccated thyroid together produce a marked increase in urine output. Addition of anterior pituitary extract to this medication caused a slight reduction in the previous high output (Heinbecker *et al.*, 1947). Thyroidectomy stops immediately the transient polyuria of simple hypophysectomy (*i.e.*, without excision of median eminence and infundibulum) and may depress that of the totally hypophysectomized dog.

The relationship between the antidiuretic hormone and the adrenals appears to be just as intimate. Adrenalectomy decreased the water exchange of hypothalamic DI (Gaunt *et al.*, 1939), which we have reason to think is identical with hypophyseal DI, and adrenocortical extract and posterior pituitary extract have been shown to have opposite effects

on urine output, concentration and output of chloride and urea in the urine of normal hypophysectomized, and adrenal ectomized animals (Anderson and Murlin, 1942). This probably needs to be qualified in view of the recent report by Deming and Leutscher (1950) on the presence of desoxycorticosterone-like (DOCA) activity (Na retention) in urinary extracts from some patients with edema due to heart failure and to nephrosis. Antidiuretic substance in the blood of rats was increased by adrenalectomy (Birnie, Jenkins, *et al.*, 1949), but found not at all in the blood of rats hypophysectomized for four to eleven days (Birnie, Eversole, *et al.*, 1949). This substance was also chloruretic and thought by the authors to be neurohypophyseal in origin. The symptoms of adrenal insufficiency have been attributed in part to antidiuretic activity both in animals (Roemmelt *et al.*, 1949) and in humans (Lloyd and Lobotsky, 1950). In the latter case a roughly reciprocal relationship was found between serum level of antidiuretic substance and excretion of urinary corticosteroids.

In 1940 Ragan *et al.* induced a polyuria in dogs with DOCA, and although pitressin did not affect fluid exchange, there was a sharp further rise in fluid exchange when pitressin was discontinued. Fluid restriction does not lead to the same degree of dehydration as in hypophyseal DI. DOCA also aggravates the symptoms of hypophyseal DI (Winter and Ingram, 1943), mainly by a tubular action. Cats appear to be little affected by DOCA as to fluid balance. DOCA administration was also followed by an increase in antidiuretic substances in the urine (Friedman *et al.*), which suggest that the adrenal may have a trophic effect on the neural lobe. Chronic administration of whole adrenal cortical extract has recently been reported by D. J. Ingle to lead to a DI-like syndrome with increased thirst (quoted by Boss *et al.*, 1950).

The posterior pituitary is only one of the regulators of body fluid exchange. Although the posterior pituitary of all vertebrates, including birds, amphibians, and teleost and elasmobranch fishes, contains antidiuretic principle, only the mammalian kidney reacts to it; therefore it is inferred that it promotes reabsorption of water from the loop of Henle and the terminal segment of the renal tubule which appears first

in mammals (Peters, 1944). Even in the most severe human DI the fluid exchange rarely exceeds 20 liters. Ranson's cats, whose normal fluid exchange was about 100 ml. per day, rarely exceeded one liter, even when histologically the gland appeared to be completely destroyed. Hence the tubular mechanism is yet 80% or more effective, since it has been demonstrated that the glomerular filtration rate (GFR) of DI is not very significantly altered (Peters, 1944). Shannon (1942) suggests that the urine of DI represents a filtrate from which water and salt have been removed in the proximal tubule and loop of Henle, but on which the final abstraction normally taking place in the distal tubule has been interferred with. As pointed out earlier, the kidney in DI appears able to excrete as much of solid matter as the normal per day, but in more dilute form, that is, requiring more water, and the antidiuretic action of pitressin is accompanied by an increase in the excretion of chloride. It seems evident that pituitary extract acts directly on the kidney, being carried by the blood stream from the hypophysis (although a central correlation has been suggested due to the lack of efficacy of pituitary extract during sleep, deep hypnosis, progressive paresis, and certain brain tumors, Sollman, 1948). Claims that secretion is by way of the cerebrospinal fluid (Cushing, 1932) have not been substantiated. The renal action of the antidiuretic principle and its hormonal character are supported by the following experiments: the perfused heart-lung-kidney preparation, and the isolated kidney, respond to pitressin by diminution and more concentrated kidney excretion of urine; the normal dog kidney whose vessels are connected to a DI dog secretes a voluminous dilute urine, which may be reversed toward the normal by injection of pitressin or by connecting the kidney vessels to another normal dog; the antidiuretic effect of pituitrin is unaffected by complete denervation of the kidney (Verney, 1926, 1929).

Verney (1946) was able to diminish urine flow by injecting hypertonic solutions of various kinds into the carotid or malleolar artery. The effect occurred much sooner after the carotid injection, and was abolished altogether by removal of the neural lobe. The effect was abolished or greatly delayed

and less pronounced when the solution was injected into the carotid artery which had been ligated superior to the point of injection and superior to the carotid sinus and body, pointing to an intracranial site of action. When NaCl was used, normal urine output returned as the elevated serum chloride declined. Hare (1947) studied the release of pituitrin as indicated by the chloride R/P ratio, stimulating its release by the injection of hypertonic saline. Release of pituitrin did not occur after stalk section, although the blood supply to the neural lobe was intact, as indicated by postmortem india ink injections. Secretion persisted after section of all nervous pathways to the diencephalon. From this he reasoned that the "osmoreceptors" which respond to change in concentration of blood solids must be in the diencephalon, perhaps closely related to the supraoptic nucleus which in turn controls by nervous means the release of pituitrin by the neurohypysis. Hare *et al.* (1944) also found that while hypertonic solution stimulates, hypotonic solution inhibits the output of antidiuretic hormone. Another group recently found that during hypertonic saline infusion, antidiuretic activity could be detected in the jugular vein blood but not in blood from the femoral vein (Ames *et al.*, 1950). Gilman and Goodman (1937) found increased antidiuretic hormone in the urine of animals after dehydration. Marx (1930, 1935) found low blood levels of antidiuretic principle during canine water diuresis and in human DI. Heller in 1937 showed that within a certain range of concentration in the blood, the amount of horomone secreted appears to be highly independent of that in the blood. The antidiuretic principle was reported to be adsorbed to some colloidal constituent to the extent of 10 milliunits per ml., and Heller postulated that the hormone must be "liberated" from its adsorption substance before it is active, such phenomenon taking place in the vicinity of the tubules after passage through the glomerular capillaries.

The physiologic state of renal function in DI, in spite of the categorical statements in the foregoing, is not entirely clearcut. According to Sollman (1948), the renal actions of pitressin consist of a brief anuria of unexplained nature, followed by a brief diuresis which is thought due to pressor action on the

efferent arteriole plus dilatation of the afferent arteriole from systemic pressor effects. The final state is a three to ten hour oliguria, without increase in glomerular filtration. The urine volume change is proportional to previous output; if low, there may be no change at all. The increased urinary concentration of urea, urate, and PSP, and doubtless other constituents following pitressin is said not to compensate for diminishing volume, that is, smaller total amounts are excreted. From sustention of this type of oliguria, hydremia and edema may occur, especially of the CNS, with rise in intra-cranial pressure; the skin actually loses water. Winer (1942) found an acute pressor effect when pituitary extract was given to patients with DI, seen as a sharp decline in GFR, a fall in renal plasma flow (RPF) variable filtration fraction (FF) all very transient and distinct from the gradual rise in clearance of diodrast over pre-injection level. His data are not conclusive, and whereas the GFR of his patients was usually a low normal with no consistent or significant change after treatment, Weisberg and McCavack (1948) recently reported finding low or low normal GFR consistently in patients with DI alone or in association with hypopituitarism, which rose consistently after treatment, sometimes to sharply supernormal levels. Actually, however, these authors found a greater response to dosage with NaCl, but in no case did they state at what stage after medication were clearances measured. Handley and Keller (1950) using dogs with DI induced by either high stalk section or by hypothalamic puncture, observed greatly reduced GFR (measured by creatinine) and RPF, a finding they had previously observed in moderately dehydrated normal dogs. Since the FF was unchanged they interpreted this result as due to a reduction of the number of functioning nephrons. The lowered rates in the DI dogs remained the same up to two years, and could be elevated to normal by saline infusions. The anterior lobe in their dogs was normal, and since White *et al.* (1947) had reported the same with simple or total hypophysectomy, they considered these changes as neurogenic in origin. White's group however failed to get such changes in two dogs with DI from hypothalamic lesions. They found diminished diodrast clearance and diodrast Tm after thyroidectomy, with still

greater depression of these functions and mild decrease in inulin clearance in very mild asymptomatic adrenal insufficiency. Thyroid and DOCA respectively increased these clearances, but AP extract was less effective in either case than in the hypophysectomized or normal dog. Hare *et al.* (1944) studied chloride excretion in experimental DI in dogs and concluded that there was neither a maximal rate of tubular reabsorption nor a renal threshold for chloride, and that pitressin affects its excretion only through its relation to water output. Dicker and Heller (1946) working on rats felt that pitressin in low doses increases both the GFR and RPF, but that in high doses it might either increase or decrease the clearance rates. In summary, it would appear that pitressin will reverse certain mild depressions of the GFR and RPF that may be present in DI, but the nature of the depression or the reversal is far from clear. Dr. May and Miss Bennett were unable to demonstrate any change in the clearance in E. J., the patient recently studied at John Sealy Hospital, although both clearances were low in the untreated state.

The posterior pituitary has been implicated with primary uterine dystocia, or inertia since the observation of the oxytocic action of posterior pituitary extract, but no definite proofs have been adduced. Clinical evidence relates largely to such cases as (a) the ones reported by Berlind (1947), three women who had difficult labor and operative delivery with subsequent postpartum shock, then secondary amenorrhea for three to 12 months associated with DI—in these it seems obvious that DI followed postpartum shock; (b) the case of Lake (1947) whose DI began during the puerperium of the first pregnancy without apparent cause, who had uterine inertia and instrumental delivery with a second pregnancy, and whose third pregnancy ended at two months in abortion. (The patient was also acromegalic, diagnosis based on course features, positive insulin sensitivity test, depression of menses, hirsute legs, absence of libido); and (c) the case of Marañon (1947) who developed DI at age 17 and subsequently had two term stillbirths with uterine inertia, one two month abortion, and one term birth complicated by uterine inertia. She also developed hypertension. Marañon states that most females with DI do not marry,

or are sterile. Soule (1937) states that women with DI frequently go into labor prematurely.

Fisher, Magoun and Ranson (1938) reviewed and criticized the literature on experimental DI—with reference to dystocia on the basis of the following criteria, which they were careful to observe in their own work:

(a) The posterior lobe of the pituitary alone must be removed;

(b) This must be demonstrated histologically at postmortem, as well as normal function of the anterior lobe;

(c) Finally a statement must be made as to behavior of the animal during labor, over and above stating simply whether or not the animal delivered.

In seven of their 80 odd cats, DI was induced during pregnancy. These cats were seen to be unable to deliver their young, or to do so only after prolonged labor lasting up to two days. Four cats died in labor and two more died a few days after delivery. Five cats showed signs of lactation, an indication of intact anterior lobe. The features were greatly elongated and often macerated, similar to the findings of Pencharz and Long in rats (1933). Four cats with lesions insufficient to cause DI delivered with normal labors, and one later came into heat. No cats came into heat after developing the permanent phase of DI. They conclude that the neurohypophysis is essential for the conduct of normal labor until proven otherwise. Haterius and Ferguson (1938) obtained a response very similar to that of pitocin by direct electrical stimulation of the pituitary stalk in puerperal rabbits. The response was unaffected by spinal transection, vagotomy, or severance of both splanchnic nerves, but was abolished by destructive lesions placed in the infundibulum. The continuous activity of the uterus in the puerperal rabbit is progressively diminished after interruption of the afferent pathways, by section of the pituitary stalk, or section of the spinal cord above L1, suggesting that the contractions are under posterior lobe control (Ferguson, 1939).

The most recent concept with reference to posterior pituitary pathology is that of Heinbecker's (1948; Findley, 1949), which correlates deficiency of its secretion with degeneration of basophilic elements of the anterior lobe and the appearance of the

so-called Crooke cells. He was led to consider Cushing's Syndrome by the observation that hypothalamic lesions caused obesity and other changes similar to those of Cushing's Syndrome. He observed four patients showing atrophy of the paraventricular nuclei in association with Crooke cells, and a fifth patient with CA of the adrenal cortex had Crooke cells but a normal hypothalamus. "The idea was then propounded that the integrity of the basophiles and the their respective end-organs is dependent upon hormones issuing from the neurohypophysis, and that depression of basophilic function results in relative hyperfunction of the pituitary eosinophiles and their respective end-organs (Fig. 1). Basophilic hypo-activity can be induced either by any neurologic event which inhibits the function of the neurohypophysis or, more commonly perhaps, by the formation in excessive amounts of other hormones physiologically antagonistic to those of the neurohypophysis" (Findley, 1949). In the latter statement, he has in mind especially that phenomenon mentioned earlier, the antagonism between adrenocortical extract and pituitrin.

Experimental evidence derives from a series of three types of operations on the pituitary as shown in the diagram (Fig. 2),

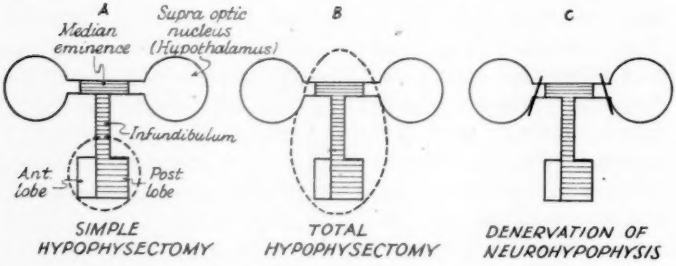

FIGURE 2. See text (modified from Findley, 1949).

A-Simple hypophysectomy, B-Total hypophysectomy, and C-Denervation of the neurohypophysis. Note that "A" represents functional removal of the anterior lobe, and "C" represents functional removal of the posterior lobe. "C" is followed after a latent period of a few months by a large decrease in the number of basophiles and degeneration and degranulation of

these few remaining. These animals show regressive changes in the thyroid, follicle cells of the ovary and the sperm cells in the male. It is concluded that the basophiles exert trophic action on these organs (Fig. 1). A comparison with the effects of operation "B" leads to the further conclusion that the eosinophiles are trophic to the heart, renal tubules, adrenal cortex, corpus luteum, cells of Leydig, and the prostate (Fig. 1). The importance of the pituitary eosinophiles in the regulation of the circulation is shown by the high urea clearance in acromegaly, and the profound drop in arterial pressure, cardiac output, renal blood flow, and diodrast Tm which follow simple or total hypophysectomy but not denervation of the neurohypophysis. Heinbecker suggests from this that growth may be largely a function of blood flow.

Observations which are not yet proved or confirmed but are essential to the integrity of the hypothesis on its present basis are the following:

(a) That a correlation actually exists between the cellular structure observed histologically and the function of the anterior lobe;

(b) That the growth and integrity of the basophiles depends on humoral influences from the neurophypophysis;

(c) That hypofunction of the neurophypophysis sensitizes the organism to adrenocortical hormone and possibly to other pressor hormones as well;

(d) And that the pituitary eosinophiles exert a profound effect upon the circulation.

Observation (c) provides a key to the most prominent application of the hypothesis made by Heinbecker, namely its relationship to the pathogenesis of diastolic hypertension. This is offered to explain the lack of finding of elevated levels of pressor substances in the bloodstream of hypertensive individuals. It is suggested that functional depression occurs as result of nervous impulses from the cortex, probably the frontal lobes, and that pressor agents are secreted, as a result of eosinophile overactivity, by the adrenal cortex and the kidney (renin). Hypertension in pregnancy is explained by progesterone antagonism

of the hormones of the posterior pituitary, which amounts to functional inhibition.

Thus, according to the hypothesis, Cushing's Syndrome might be produced in any of three ways, by a) overproduction of steroid hormones, *i.e.* from the ovary or the adrenal, which are antagonistic to the effect of pituitrin; by b) a destructive lesion of the hypothalamus, or by c) functional inhibition of the neurohypophysis, presumably from cortical levels.

Evidence is cited to support the antagonistic behavior of DOCA, progesterone, renin, and epinephrine toward pituitrin, and considerable collateral data is adduced to tie in diabetes mellitus, arteriorsclerosis, obesity, muscular weakness, osteoporosis, sexual impotence, chiefly from the standpoint of adrenal overactivity. The question is raised as to the lack of frequency with which DI is associated with hypertension; it may be actually not uncommonly associated with one of several of the above symptoms. Heinbecker's series of patients with DI had a relatively high incidence of hypertension, and Findley's series of five included three with tension greater than 140/90. Heinbecker further pointed out that in DI there is often some degree of injury to the anterior lobe as well as the posterior, and it should be remembered that, hypothetically speaking, the paraventricular nuclei are under greater suspicion in connection with the above phenomena than the supraoptic nuclei which are related to DI.

Following the same line of reasoning Heinbecker (1949) studied a group of patients with toxic goiter. On the basis of histological studies he postulated that hyperthyroidism accompanied by proptosis of the eyes (exophthalmic goiter) is due to overactivity of the paired paraventricular nuclei, operating through the same system described above. Other types of hyperthyroidism could not be correlatetd in this manner.

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ANTIBIOTICS AND THE ADDED YEARS*

ROBERTSON PRATT

Therapeutic use of antibiotics may be doing more to increase the life span of man than any single product of biologic or medical research of the last 150 years. The impact that discovery and widespread use of penicillin, streptomycin, and other antibiotics is having on the social and economic structure is not generally recognized.

While solving one problem and providing means for technologic advance, research often gives rise to important new problems. These are not always scientific; sometimes they are social or economic. Pharmaceutical and medical research are not exceptions. They are directed primarily toward improving health and adding years to man's life. But in accomplishing this, they too sometimes add to the complex socio-economic problems that confront humanity today. The advent of antibiotic therapy has emphasized this. What is the value of adding years to man's life unless simultaneously life can be added to his years?

Since the time of the Industrial Revolution in the late 18th Century there has been much discussion of the social problems created by application of scientists' technologic knowledge. This discussion has continued through two devastating World Wars and recently has been intensified by development of the atom bomb and plans for the hydrogen bomb. Recent concern in official circles and among the public has focussed attention on the dramatic and potentially devastating physical forces that man has learned to release but only partially to control. This has tended to obscure another equally important but less

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*From the University of California College of Pharmacy, The Medical Center, San Francisco, California. Condensed from an address delivered August 2, 1950, at a symposium on Newer Aspects of Pharmacy held at the University of California College of Pharmacy, where Dr. Pratt is director of the Antibiotics Research Laboratory. Dr. Pratt is senior author, with Jean Dufrenoy as collaborator, of the recent monograph, "Antibiotics," 255 pp., 61 figs., Philadelphia, J. B. Lippincott Co., 1949. For another treatment of social aspects of antibiosis see "Armageddon of the Microbes" by the same author (this Journal, 1949, 7(1):12–21). Received for publication July 18, 1950.

dramatic problem that has arisen from men's learning to control certain insidious forces of nature, namely, a number of infectious diseases. Put bluntly, this is the problem caused by an abnormal and disproportionate shift in the structure of our population toward an increasing number of people in the upper age groups. The trend toward an increasing proportion of older individuals in the population has been developing for years, largely as the result of improved health care and sanitation, both of which have arisen from fundamental biologic research. Until recently, the change has been relatively slow, and there has been time for the economy to make at least partial adjustment. However, in the last decade the problem has been accentuated by the general therapeutic use of antibiotics, although the full social and economic impact of these life-saving drugs will not be felt for several years—perhaps not for two or three generations.

It seems paradoxical that in learning to control the economically and socially destructive forces of veneral diseases, tuberculosis, plague, typhus, pneumonia, and a host of other infectious maladies, scientists should have generated new social problems.

On the one hand, antibiotics, by reducing necessary periods of hospitalization and quarantine and, therefore, permitting wage earners to return to work sooner than previously, tend to relieve the immediate financial and psychologic strain on families in times of stress due to illness: on the other hand, simultaneously they create a problem of the gravest consequences for the future. This problem has many interrelated and almost inextricably associated ramifications. It is not always possible to completely disentangle the threads connecting its several parts. For convenience, however, the question may be considered from two general points of view—one social, the other economic.

Due to declining birth rate and improved medical care, we are rapidly becoming a nation of middle-aged and elderly people. This trend, which has been apparent for some time, has been accelerated markedly since antibiotics first became available for civilian use. In 1900 the average length of life in the

United States was 49.2 years; in 1945 it was 65.8 years; and in 1948 it was 68 years. These figures are highly significant. In the first forty-five years of the twentieth century the average life span increased 16.6 years. It rose 2.2 years between 1945 and 1948. In other terms, the average increment in life span was 4.4 months per year for nearly half a century. Then, suddenly, in so short a time as three years, the average increment in life span doubled to 8.8 months per year. It seems entirely plausible to attribute this sharp increase principally to antibiotics. Penicillin and small amounts of streptomycin first became available for general civilian use in 1945. That very year the increase began. Naturally, the mere fact that events concur in time does not necessarily imply that there is a cause and effect relation between them. However, when a natural process proceeds at a uniform rate for almost half a century and then in the brief span of three years departs precipitously from the established pattern, there must be an underlying cause. In this instance, it seems logical to ascribe the change to antibiotics, since no other pharmaceutical or medical developments during the period fit into the picture. Despite improved diagnosis and treatment of the two great killers heart disease and cancer, deaths from these causes continued to increase. Only in the area of infectious diseases amenable to antibiotic therapy has there been a sharp decline in death rate. Sulfa drugs were introduced into general use a decade earlier and can be ignored as an important factor in the present marked departure from the previous pattern. Antibiotics appear to be the only new factor in our biologic environment during the period of pronounced change from the previous course of events.

During the period in question, *i.e.*, 1945–1948, only two antibiotics, penicillin and small amounts of streptomycin, were available for general use in treating systemic infections. Now, production of these two drugs is sufficient to satisfy all domestic needs. Moreover, there are also available several newer antibiotics which are useful for treating typus, whooping cough, some types of influenza, and a number of other infectious diseases not amenable to therapy with penicillin or streptomycin.

Therefore, greater increases in life span can be expected in the future.

What does this mean for future economy? It means that a decreasing proportion of the population, namely the industrially active and productive age groups, may need to support an increasing proportion of elderly people who, through no fault of their own, are denied the privilege of working and who, industrially speaking, are non-productive. Whether these people are supported on an individual or family basis by relatives or are supported by social security or other programs financed partly or wholly by taxes is immaterial. The fact remains that a diminishing proportion of the population may be faced with the necessity of supporting an increasing segment.

Estimates by authorities in the field of population statistics predict that by the year 2000 (just a little over two generations hence) approximately 40 per cent of the population of the United States will be 45 years of age or older and that approximately 13 per cent will be more than 65 years of age. According to the most conservative estimates, between fifteen and twenty million Americans will be over 60 at that time. When these estimates were published (1946) the profound effects of antibiotics on the structure of our population had not yet become apparent. Therefore, it is quite likely that the actual figures will be considerably higher than the estimates.

Realization of these facts brings us back to the question—"Of what value is it to add years to one's life unless concomitantly life can be added to one's years?" Man is essentially an active creature. He likes to be busy, or at least reasonably so. Yet many business concerns are loath to hire new workers over 40 or 45 years of age and in some instances to retain employees over 55 or 60 years of age. Who is going to provide the necessities of a comfortable life for them? The problem has begun to receive some attention from leaders of management and of labor. A small step toward solution of the problem is being made in some current labor contracts providing for retirement benefits. Regardless of the adequacy or appropriateness of this solution, such arrangements, at best, can care only in a limited way for a small minority of the population as a

whole. At present, no provision is made for vast numbers of clerical and other white collar workers, for agricultural workers, or for many transients who work only on a day to day basis.

Over and above the economic problems arising from a rapid change in the population structure with regard to age distribution are serious medical and social problems which may be even more important. Prominent among these are matters of physical and mental health.

The diseases of childhood, adolescence, and early adult life are largely infectious in origin. Many, if not most, of them can be avoided by proper prophylaxis. In the older age groups a different situation exists: the most common incapacitating ailments are not infectious. Although methods of diagnosis and treatment are being improved constantly, there are not known any universally effective prophylactic measures against cancer, many heart disorders, and organic disturbances that arise from degenerative changes in the tissues. The major source of disability in the elderly is internal and not external. Treatment is largely therapeutic and not prophylactic. Thus by markedly prolonging the average life span, universal use of antibiotics may tend to focus medical attention on the degenerative diseases that are most prevalent after middle life.

Equally important as and intimately related to the physical condition of the elderly individual is his mental outlook on life. Often it is devastating to the morale and spirit of a man or woman in good physical condition to be retired from business just because of age. Indeed, often such individuals as a result of their years of experience and acquired skills, are in a better position to make notable contributions to society than their younger associates. Most people like to feel that they are necessary or useful. How to abate and avoid the mental let-down that often develops rapidly and results in premature senescence when useful occupation is prohibited are major problems facing medico-social workers and psychiatrists today. But this is not a task for the professional man alone. In the words of Robert Browning.

"The common problem, yours, mine, every one's
Is—not to fancy what were fair in life
Provided it could be,—but finding first
What may be, then find how to make it fair
Up to our means."

The old pattern—youth, middle age, "retirement"—is incompatible with the trend of our society. A new cycle of life is in order. Research has given us more years in which to live. Now we must solve the problem of giving Life to those years.

A SPECIFIC TRANSPLACENTAL EFFECT OF URETHANE IN MICE*

JOHN G. SINCLAIR

Ethyl carbamate has been used therapeutically since 1885. On the European continent it was used as a sedative for the insane and in the control of hysterias. In England Sieveking (1886) reported its successful use in controlling intractable pain from many severe conditions, including massive internal cancers. Its great advantage lay in maintaining normal respiration and normal blood pressure. The beneficial effect from one hypnotic dose often lasted four days. Barcroft in 1939 noted, however, that even light urethane anesthesia during birth labor excessively depressed the fetal respiratory center in the goat.

Modern study of the action of urethane has disclosed some interesting properties. Weinstein (1945) reports that its bacteriostatic action promotes wound healing. Bodine (1949) says it is a mild metabolic depressant for blocked embryonic cells. Hawkins (1925) and Murphy (1946) said that in rats it raised CO_2 levels and caused alkalosis. It apparently blocks thymonucleic acid synthesis in tissue culture. This mechanism also suppresses marrow reproduction almost specifically. The effects are very similar to X-ray effects according to Paterson (1946) and Bucker (1947). It remains one of the most potent of the large list of related carbamates. Skipper *et al.* (1948) have made an extensive study of the whole group. Urethane is said to block the dehydrogenase system except for about 30 per cent which seems to be immune or resistant in the Bodine's (1950) study of embryonic cells. On bioluminescence it acts as a class II inhibitor causing irreversible protein denaturation (Johnson, 1945). Tracer techniques applied to its parts indicate that the drug is completely eliminated normally within 24 hours but that in the presence of large tumors it may be retained longer (Mitchell, 1940). Everything pharmacologically points

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to a relatively harmless drug if used in small doses. It is not, however, without some dangers.

In 1943 Nettleship had seen in mice that as a result of repeated 25 mg. injections of urethane lung tumors had appeared in strains free from these tumors. Guyer in 1948 observed in rats that animals subjected to urethane anaesthesia and then kept for some months developed multiple lung tumors (see also Rosin, 1949). Several recent studies show that as little as 1 mg. daily will greatly hasten the formation of pulmonary tumors in strains that develop such tumors spontaneously. The tumors are adenomas and no other reaction appears at the site of injection nor at the site of urethane crystal implantation (Henshaw, 1945). Other agents will produce the same kind of tumor (Larson, 1946). He also found that 25 mg. injected into mouse mothers 24 hours before parturition produced lung tumors in 100% of the young within 6 months. Tumor induction was advanced in proportion to the dose given at one time. Noble (1948) found that if combined with zinc acetate the urethane produced sarcomas. Sinclair (1949) found that fairly massive amounts of urethane (300 mg. in three weeks) produced, five months later in strain C3H, very large skin tumors all of which were adenomas. A hyperplastic rebound of the lymphoid tissue was also noted in these mice. Guyer (1947) studied the effect of urethane on the suppression of mitosis in the cornea of the rat and found a reduction for a short period. The picture is wholly different from that produced by colchicine. No active phase is blocked. The similarity of its action to that of X-rays is emphasized when they are used together. Henry (1949) showed that under urethane anesthesia laboratory animals are easily killed by sublethal doses of X-rays. There is also danger from extreme depression of marrow reproduction and lesions from this effect were reported by Escola (1949). Recently because of its possibilities as an adjuvant it has been combined with other drugs. Peple (1949) reported two deaths from such a combination with penicillin. The Food and Drug Administration last March seized a shipment of urethane syrup because of its potential dangers.

History of the Present Experiments. Because of our experience with the tumor producing potentialities of urethane and

its physiological action we felt that it might be useful as a tool to reach across the placenta at all stages of development and depress various primordia at critical moments. We were given a Public Health Service research grant. Two medical students, Bob Eckert and Irving Watson, were trained in the care and recording of a mouse colony by Dr. Marthella Frantz. Their interest, initiative and excellent service have contributed much to the outcome.

The mouse colony was started from a C57 black stock received from Dr. A. Kirschbaum and a CBA stock from M. Frantz. We decided that using CBA females and C57 males would produce a hardy litter. The administration of urethane varied as experience dictated. Rearing the stock itself in the summer in Galveston presented considerable difficulties. Reproduction stopped and hair was lost even using Purina Fox Chow as food base with grass seed tops at intervals as supplement. Finally we added a vitamin complex called Tronic compound by Sharp and Dohme because it was miscible with the drinking water. This brought the colony back to full vigor.

We started on the assumption that urethane could be given in drinking water as I had done successfully with the C3H stock in 1948. Henshaw (1945) and Larson (1946) had also used this method. We found, however, that neither of these stocks would touch the treated water even when the concentration was cut to 1 per cent. It could not be disguised by saccharine nor by alcohol. The mice would die rather than drink. Then we adopted a 3 per cent solution and injected 1 cc. or 30 mg. subcutaneously. We found that no pregnancy occurred and if the dose were repeated every alternate day the mice died after the fourth or fifth injection. We attempted to determine precisely when insemination occurred by studying vaginal smears for the onset of estrus and vaginal plugs to indicate successful insemination. Vaginal smears proved quite unsatisfactory in this stock and vaginal plugs were poor evidence. Any injection during the first week after insemination prevented development or at least implantation. Since repeated 30 mg. doses killed the mothers we dropped to 15 mg. and started the injections on the 7th or 8th day after the appearance of the evanescent vaginal plug. Some pregnancies resulted

and were followed to parturition but we obtained no litters for study. The mothers ate all their young during the first day.

At this time we were fortunate in securing an albino stock from Dr. Isabel Blount. It had been selectively bred for twenty generations from the Carworth Farms general purpose stock and was known to be hardy and tumor free. This stock could stand the warm weather and continue to breed. It also tolerated the drug well. In this stock we found that vaginal plugs were easily seen so that we had a definite landmark for our first injections a week later. We continued the 15 mg. injections every second day. Some of the litters from this stock also were aborted during pregnancy but some survived and were taken from the mothers at the nineteenth day. The whole litter was fixed in utero in Zenker's solution. Each embryo was embedded separately and they were cut serially.

It was in the first embryo of this series that a lethal anomaly was observed. It is shown in Figure 1. Starting along the neck dorsally, skin is found to be continuous with choroid plexus of the fourth ventricle. The entire brain is exposed to the amniotic vesicle without cover and it is not closed so that the lateral ventricles are spread open just above the eye. The eye itself is quite developed except for rudimentary lids. The other tissues of the embryo are fully differentiated and ossification of ribs has started. Examination of other embryos of this litter showed the same anomaly.

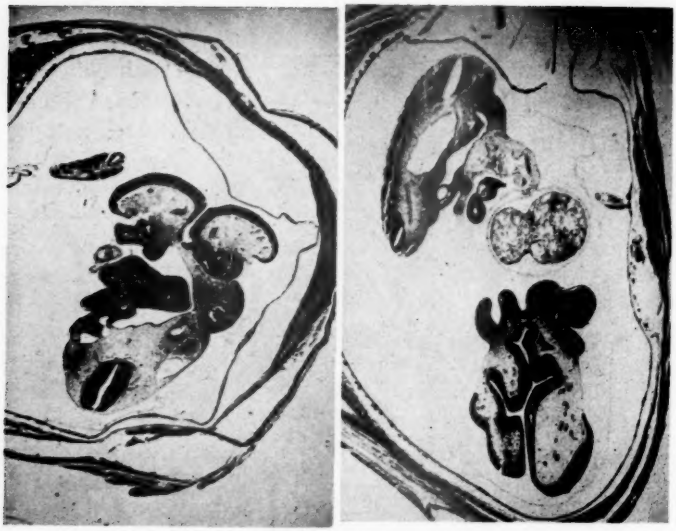
It is obvious that the failure of the central nervous system to close involves a block early in development; in fact, early in the 8th or late in the 7th day. We now began to limit injections to two or three during the first week of development after implantation and then took the litters. Some of the members of these litters were found to be dead in utero but of the remainder we found that embryos of the 15th and 16th days displayed the same anomaly in very diagrammatic form as seen in figures 2 and 3.

Attention was now directed to the litters resulting from the initial injection on the 8th day after it was too late to influence the closure of the nervous system. These embryos show a second anomaly. A zone lying between the germinal zone and

FIG. 1. Section of 19 day mouse embryo from mother injected with 15 mg. of urethane on alternate days, starting the 7th day of pregnancy. Relatively normal except for the exposed brain plate which has not closed and is continuous with skin.

the differentiated cells of the basal plate of the brain and spinal cord show intense nuclear degeneration. This column of cells at a special stage of differentiation starts in the midbrain and tapers off in the less differentiated lower spinal cord. A view of the degeneration in the brain and spinal cord is given in figures 4 and 5.

We are in the midst of the experiment and have not analyzed the effects further. There may be other less obvious effects of the drug. We are reducing the dose of urethane to its minimum effective dose given at a specific time. The minimum to date has been two 15 mg. injections two days apart. Concentration in the fetus must be considerably lower than that in the mother if the mother's eliminating mechanism works as rapidly as is believed.



FIGS. 2 AND 3. Mouse embryos of the 15th and 16th days of gestation from mothers injected with 15 mg. of urethane on the 7th and 9th days of pregnancy. The brain is wide open and continuous with skin. The spinal cord is closed. Eye formation is normal.

We have no solution to offer as to the mechanics of failure to close the brain while the spinal cord closes as usual. Mitosis is not blocked very long, judging by the number of figures seen in the germinal zone. We feel that the experimental series as a whole are somewhat behind the controls in rate of development. Tissue differentiation is generally quite normal. Many anomalies have been produced as a result of genetic defects and from vitamin deficiencies. This anomaly is not among them (Snell and Pickens, 1935 and Warkany, 1948). Gilman (1948) saturated the tisues with trypan blue and produced a number of anomalies apparent at birth. The anomalies do not include this one and in general were produced at a later stage.

The present paper indicates that the action of urethane, a relatively innocuous drug, when given in subhypnotic doses at critical periods in gestation can produce relatively severe effects even across the placenta. The specificity of the effect suggests

FIG. 4. Section of the hindbrain of a 15 day embryo mouse from a mother injected with 15 mg. of urethane on the 8th and 10th days of pregnancy. The basal plate shows complete nuclear degeneration.

also that such drugs blocking or accelerating specific links of the metabolic and synthetic processes may be used profitably in experimental embryology.

CONCLUSIONS

1. A subhypnotic dose of urethane (15 mg.) injected subcutaneously into pregnant mice of two strains on the 7th day of pregnancy prevents closure of the brain, but not spinal cord, of each embryo. Fetal death results from a 30 mg. dose.

2. Other developments and tissue differentiations are not obviously affected, though judgment on this point must be reserved. The repeated injection of 15 mg. on alternate days results in many fetal deaths and abortions.

3. The brain anomaly has appeared in all members of a litter and in two strains of mice normally free of anomalies. It is

FIG. 5. Section of the spinal cord of the same embryo as figure 4. The degeneration of the incipient motor cell column is continuous through brain and spinal cord.

compatible with normal growth and differentiation of most tissues and organs until the time of birth.

4. Defective living litters are eaten by the mothers within 24 hours.

5. A subhypnotic dose injected on the 8th day produces a degeneration of the incipient motor cells of the whole nervous system. This also is found in many embryos though in the same uterus the anomaly may appear in one embryo while the adjacent embryo is dead and shows post mortem changes.

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CALLING ATTENTION TO:

Items of Possible Interest to Our Friends

September, 1950

General: EN Andrade well biographizes Robt Hooke (1635–1703) (*Proc Roy Soc B* 137/153/50). In classifying human races by blood groups, WC Boyd finds no evidence for inherited racial superiority or inferiority (*Science* 112/187/Aug 18/50). LR Grote offers tribute to F Volhard (1872–1950) (*Arzt Forsch* 4/381/July 25/50). L Hogben pictures *From Cave Painting to Comic Strip* (Chanticleer Press, NY, 50, 330pp $5). RD Huntoon & V Fano wisely propose atomic definitions for primary standards of measurement, as atomic meter equal to 1,831,249.2 wave-lengths from 5460.7532A line of mercury-198 in standard air, & atomic second as time required for $238{,}701 \times 10^5$ oscillations of the 23,870 Mc or 3.3 line of NH_3 (*Nature* 166/167/July 29/50). R May discusses *The Meaning of Anxiety* (Ronald Press, NY, 50, 376pp, $4.5). WV Mayneord offers applications of nuclear physics to medicine (*Suppl* 2, *Brit J Radiol*, 50, 289pp). JC Meakins, HE Hoff & P Sekelj (*Am Heart J* 40/163/50) show historical relations of W Einthoven's report on electrical axis of heart (*Lancet* 1/853–861/1912) & Einthoven, G Fahr & deWaart's exposition (*Arch ges Physiol* 150/275–315/13). A Montagu reports UNESCO's statement on race (*Sat Rev Lit* Aug 19/50, p8). A Rapoport & Co give biobibliography of AHS Korzybski (1879–1950) *Etc* 7/163/50). PB Sears discusses *Charles Darwin as a Cultural Force* (Scribner, NY, 50, 134pp, $2). C Smith answers ? how good is your family doctor? (*Atlantic* August 50, p43). HB Vickery relates origin of word "protein" by Berzelius in 1838 (*Yale J Biol Med* 22/387/50). A Vogel describes how diuretic action of organic mercurials were discovered by him in 1919 (*Am Heart J* 39/881/50). WH Popsy Welch (1850–1934) is eulogized by top-flying DW Bronk, EW Goodpasture, AW Freeman, RH Shryock, A Gregg & A Flexner (*Suppl Bull Johns Hopkins Hosp* 87/1–54/50). N Cybernetic Wiener alarmingly views *The Human Use of Human Beings* (Houghton Mifflin, Boston, 50, 241pp, $3). JH Wills echoes others in considering scientific position of pharmacology (*Sci Monthly* 71/91/50). JT Winchell criticizes semantics of JB Conant's educational theories (*Etc* 7/243/50). MC Winternitz is properly saluted by colleagues (*Yale J Biol Med* 22/467–774/50).

Anesthesia: KAC Elliott & Co note anesthesia raises brain acetylcholine (*Am J Physiol* 162/469/50). DF Marsh offers comprehensive comparison of aliphatic ethers (*Anesth* 11/455/50). Our HC Slucum, BM Cooper & CR Allen describe control of respiration in anesthesia (*Anesth* 11/477/50).

Fundamental: JM Carlisle & Co offer neat exhibit on cortisone (*Postgrad Med* 8/113/50). S Dahl finds serum magnesium levels in healthy humans averaging 2.1–2.2 mgm% (*Acta Haematol* 4/65/50). A Fessard

& J Posternak give detailed analysis of synaptic transmission (*J Physiologie* 42/319–445/50). HE & HC Harrison find rapid exchange of calcium between bone & body fluids increased by calciferol & Ca uptake by skeleton no measure of new bone salt deposition (*J Biol Chem* 185/857/50). AV Hill shows heat production precedes mechanical movement in muscular contraction (*Proc Roy Soc B* 137/268/50). RM Hodges shows average strontium content of human bone to be 0.024%, & lower in fetel bone & higher in 1 case of hydrocephalus (*J Bio Chem* 185/519/50). JM Hundley describes achromotrichia from copper deficiency (*Proc Soc Exp Biol Med* 74/531/50). MH Maxwell & Co show nothing unique about juxtamedullary renal circulation in man (*Am J Med* 9/216/50). RA McCance discusses renal physiology in infants (*ibid* p229). M Maruyama describes parotid fraction which lowers blood calcium (*J Biochem* 37/1/50). Where's the Howells reference in HV Meredith's study of birth order & body size? (*Am J Phys Anthro* 8/195/50). H Moussatche shows CO_2 inhibits neurone activity by lowering intracellular pH (*Arch Internat Physiol* 57/399/50). LH Newburgh offers *Physiology of Heat Regulation & the Science of Clothing*, with not even a smile (Saunders, Phila, 50, 457pp $7.5). R Osmond & JS Robertson describe photoelectric method of estimating red cells & hb (*Med J Austral* 2/36/July 8/50). HM Patt & Co find systeine significantly reduces blood disturbances caused by radiation (*Blood* 5/758/50). VR Potter discusses *Enzymes Growth & Cancer* (CC Thomas, Springfield, Ill, 50, 64pp, $2). JM Steel & Co find 45% body weight as water in females & 53% in males, with ⅔ in cells (*Am J Physiol* 162/313, 318/50). M Viscontini discusses antivitamine activity (*Schw Med Wchnschr* 80/763/July 22/50). SA Waksman summarizes *The Actinomycetes, Their Nature, Occurrence, Activities & Importance* (Chronica Botanica, Waltham, Mass, 50, 221pp $5). Our DM Whitney, L Anigstein & DW Micks note antibacterial action of hydrolyzed red cells (*Proc Soc Exp Biol Med* 74/346/50). DW Woolley edits symposium on *Anti-metabolites* (*NY Acad Sci* 52/1197–1378/50).

OCTOBER, 1950

CLINICAL: M. Boigey analyses physiology of cold bath and dangers therefrom (*Presse Med* 58/854/July 29/50). Our JY Bradfield & MR Hejtmancik indicate rheumatoid arthritis & rheumatic fever are allergic in origin (*Arch Int Med* 86/1/50). E Braun-Mendenez shows increased NaCl retention in hypertension (*Rev Soc Argent Biol* 26/16/50). WP Deiss & PP Cohen suggest low p-aminohippuric acid synthesis in primary liver disease as diagnostic test (*J Clin Invest* 29/1018/50). H Dubois-Ferriere says Na Naphthylamine sulfonate is useful hemostatic agent (*Schw Med Wchnschr* 80/861/Aug 19/50). MM Gertler & Co show high serum cholesterol & phospholipids in patients with coronary disease (*Circula* 2/205/50). JW Gofman & large Co show relation of big lipoprotein molecules to atherosclerosis (*Ibid* p 161). H Glorieux & Co offer

symposium on medical protection against modern weapons (*Presse Med* 58/902–919/Aug 19/50). FHK Green's committee finds no value in antihistaminics in treating common colds (*Brit Med J* 2/425/Aug 19/50). T Greiner & Co by new daily report evaluation method show khellin no better than lactose in controlling cardiac pain of angina of effort (*Am J Med* 9/143/50). M Kelly offers methods for preventing deformities of rheumatic disease (*Med J Austral* 2/1/July 1/50). P Klemperer well discusses concept of collagen diseases (*Am J Path* 26/505/50). RK Kracke's study of hereditary spherocytic anemia in negroes appears posthumously (*Blood* 5/793/50). BVA Low-Beer describes *Clinical Use of Radiactive Isotopes* (CC Thomas, Springfield, Ill, 50, 436pp, $9.5). PS Menon & Co indicate phrynoderma is manifestation of essential fatty acid deficiency (*Indian J Med Res* 38/173/50). LJ Meduna revives our old work on CO_2 treatment of schizophrenia (*Calif West Med* 31/21/29) as book: *Carbon Dioxide Therapy: Neurophysiological Treatment of Nervous Disorders* (CC Thomas, Springfield, Ill, 50, 248pp, $5). W Penfield offers *Epileptic Seizure Patterns* (CC Thomas, Springfield, Ill, 50, 144pp, $2.5). Our A Ruskin & Co show "Thiomerin" is mercurial diuretic of choice for parenteral administration (*J Lab Clin Invest* 36/1/50). LE Schaefer & Co find Na gentisate effective in acute rheumatic fever without side action (*Circula* 2/265/50). H Scholer & M Springenschmid describe phonecardiography (*Schw Med Wchnschr* 80/807/Aug 5/50). R Turner & S Saville suggest methonium cmpds for hypertension (*Lancet* 2/353–358/Sept 2/50). J Wortis discusses *Soviet Psychiatry* (Wms & Wilkins, Balt, 50, 329pp, $5). O Zima, F Eichholtz, A Sauer & Co find "Egressin" (an alkyl phenyl carbamate) useful in pin-worm infection, without untoward effect (*Schw Med Wchnschr* 80/734/July 15/50).

Basic Stuff: CH Best & Co by dose-effect studies confirm our WH Griffith (23/91/42) that lipotropic requirement is related to caloric intake & that choline has strong lipotropic effect under all dietary conditions (*J Biol Chem* 186/317/50). RT Clark shows mice oxidize CO to CO_2 at rate of 0.28 cmm/gm/hr (*Am J Physiol* 162/560/50). G Dahlberg wisely discusses probability in medicine (*Acta Gen Statist Med* 1/313/50). SK Elster finds ascorbic acid deficiency does not reduce collagen content of tissues (*J Biol Chem* 186/105/50). LA Lewis offers Electrophoresis in Physiology (CC Thomas, Springfield, Ill, 50, 72pp, $1.85). CS Hanes & Co show role of glutathione in synthesis of peptides (*Nature* 166/288/Aug 19/50). LJ Lorch & JF Danielli achieve transplantation of nuclei from cell to cell (*Nature* 166/329/Aug 26/50). F Schrader & C Leuchtenberger offer cytochemical analysis of functional interrelations of various cell structures (*Exp Cell Res* 1/421/50). C Terner & Co suggest 1-glutamate functions in potassium transport (*Biochem J* 47/139/50).

General: R Carnap finds *Logical Foundations of Probability* (Univ Chicago Press, 50, 680pp, $12.5). A Cooke records *A Generation on Trial: USA vs Alger Hiss* (Knopf, NY, 50, 300pp, $4). S Epstein gives history of celebrated American leg amputees (*Angiol* 1/351/50). ML Johnson

recommends discussion methods in preclinical teaching (*Lancet* 2/313/Aug 26/50). MA Maxwell describes rise & fall of Alcoholics Anonymous of century ago, the Washingtonians (*Quart J Alc* 11/410/50). GP Meredith & Sir Henry Cohen defend lecturing in medical teaching (*Brit Med J* 2/475–478/Aug 26/50). GE Moloney & Co discuss social aspects of appendicitis (*Brit J Surg* 38/52/50). Wise OHP Pepper urges Greek & Latin learning for appreciation of medical terminology (*Proc Am Philo Soc* 94/364/50). L & G Piternick & Co translate Mendel's letters & reports of rediscovery (*Genetics*, Suppl Vol 35, No. 2, Pt. 2, Sept 50). EH Von Foerster edits trans 6th Conf on Cybernetics: *Circular, Causal & Feedback Mechanisms in Biological & Social Systems* (Macy Founda, NY, 50, 209pp). PD White notes historical delays in applying cardiac knowledge (*J Med Asso Alabama* 20/66/50).

NOVEMBER, 1950

GENERAL: KA Bisset discusses evolution of bacteria & significance of spores (*Nature* 166/431/Sept 9/50). J deBoer in analysing action of coniine concludes death of Socrates (469–399 BC) was due to combination of hemlock & opium (*Arch Internat Pharmaco Therap* 83/473/50). BJ Lindberg reviews psycho-infantilism (*Acta Psych Neurol Suppl* 61, Copenhagen, 50, 126pp). NH Mackworth offers *Researches on Measurement of Human Performance* (Sp Rep Med Res Counc 268, 50, 4s). JH Means indicts AMA's political policies (*Atlantic*, Oct 50, p 57). E Nrodlander analyses male sterility (*Acta Obs Gyn Scand* 30/Suppl 7/220pp/50). LS Penrose is optimistic about reducing propagation of unfit (*Lancet* 2/425/Sept 30/50). AH Schultz describes physical distinctions of man (*Proc Am Philos Soc* 94/428/50). A Vogl tells how he discovered (*Wien Klin Wchnschr* 48/400/1935) diuretic action of organic mercurials (*Am Heat J* (39/881/50). DW Winnicutt analyses meaning of word "democracy" (*Human Relations* 3/175/50).

CLINICAL: FA Brandt finds radiation harmful in histoplasmosis (*S Afr J Med Sci* 15/1/50). SM Feinberg & Co offer *The Antihistamines: Their Clinical Application*, Yearbook, Chicago, 50, 291pp, $4). E & J Frey offer *Die Funktionen der Gesunden & Kranken Niere* (Springer, Heidelberg, 50, 168pp, M20). I Lindgren reviews neurosurgery of angina pectoris (*Acta Med Scand* 138 Suppl 243, 50, 203pp). GC Meirsman-Roobroeck notes protective action of myocardial depressants (parpanit, dibenamine, acetylcholines) against adrenin-release ventricular fibrillation (*Arch Internat Pharmaco Therap* 83/353/50). A Perdrup analyses burn treatment with sulfa water ointment (*Acta Chir Scand* 100/136/50). WB Shelley & Co consider etiology of anhidrosis (*Med* 29/195/50). SO Wschwartz & L Ehrlich review relation of polycythemia & leukemia & warn of danger of latter from irradiation therapy of former (*Acta Haematol* 4/129/50). A Videbaek considers prognosis in polycythemia vera (*Acta Med Scand* 138/179/50). DW Watkins & Co say W Kempner's rice diet (*Ann Int*

Med 31/821/49) reduces serum cholesterol fractions in hypertension (*Am J Med* 9/428, 494/50).

FUNDAMENTAL: F Bergman & Co offer structural features of acetylcholinesterase which are involved in inhibition (*J Biol Chem* 186/693/50). CD Carpenter & Co say allyl pytrethrins (alletrins) are as satisfactory & safe as pyrethrins for insecticidal use (*Arch Ind Hyg Occup Med* 2/420/50). H Dahlstrom discusses basal metabolism, extracellular fluid, fat combustion & thyroid hormone (*Acta Physiol Scand* 21, Suppl 71, 50, 80pp). HL Davis edits symposium on mechanism & evaluation of antiseptics (*Ann NY Acad Sci* 53/1–219/Aug 30/50). L Eldjarn shows slow metabolism & elimination of tetraethyl thiuramdisulfide (Antabuse), suggesting lower dosage, & action by reduced from (*Scand J Clin Lab Invest* 2/202/50). O Friberg & Co show parallelism between urinary excretion of water, ammonia & potassium (*Ann Med Exp Biol Fenn* 28/144/50). I Geschwind & Co show growth hormone maintains liver ribonucleic acid (*Arch Biochem* 28/73/50). RJ Goldacre & IJ Lorch reveal protein molecule folding & unfolding in ameboid movement & osmotic work (*Nature* 166/497/Sept 23/50). H Granados finds new growth promoting factor in human saliva (*Acta Path Microb Scand* 27/501/50). BE Hall suggests enzymatic character of Castle's intrinsic factor for erythrocyte maturation (*Brit Med J* 2/585/Sept 9/50). P Holtz & G Kroneberg discuss oxidation of adrenalins & arterenols (*Biochem Ztschr* 320/335/50). CS Keefer & GL Hobby edit symposium on terramycin (*Ann NY Acad Sci* 53/221–460/Sept 15/50). I Kruse & H Dam find cobra venom inactivates thromboplastin (*Biochim Biophys Acta* 5/268/50). P Moser analyses osmotic resistance of young human red cells (*Ztschr Klin Med* 146/593/50). GAG Mitchell reviews nerve supply to kidneys (*Acta Anat* 10/1/50). WLM Perry discusses *Design of Toxicity Tests* (Med Res Council Sp Rep 270, 50, 76pp, 1s6d). Proceedings 1950 fall meeting (Boston, Nov 13) Am Soc Pharmacol Exp Therap raise question about publication in mimeo form: why not publication & free distribution from research labs, with expense as part of the research effort? AB Roy gives evidence on tocopherols in relation to cholesterol degrading hormone (from anterior pituitary) & carcinogenesis (*Schweiz Med Wchnschr* 80/1030/Sept 23/50). Note Symposium on heart muscle energy, proteins, & submicroscopic structure (*Scand J Clin Lab Invest* 2242/256/50). G Vanden Heuvel-Heymans shows dextran restores physiological mechanisms of blood pressure homeostasis (*Arch Internat Pharmaco* 83/308/50). J Zacek & M Rosenberg find x-rays destroy red cell membrane lipoproteins (*Biochem Biophys Acta* 5/315/50).

HAPPY(?) HOLIDAZE, 1950

MIDCENTURY MADNESS: J Boswell's *London Journey 1762* is autoanalytic (McGraw-Hill, NY, 50, 370pp, $5). J Dos Passos lectures on *The Prospect Before Us* (Houghton-Mifflin, Boston, 50, 375pp, $3.75). JS Finch appreciates *Sir Thomas Browne: A Doctor's Life of Science and*

Faith (Schuman, NY, 50, 319pp, $3.5). MT Gnudi & JP Wegster offer *Life & Times of Gaspare Tagliacozzi: Surgeon of Bologna, 1545–1599* (Reichner, NY, 50, 562pp, $15). JO Hirschfelder edits *The Effects of Atomic Weapons* (McGraw-Hill, NY, 50, 456pp, $3). EH Hume describes *Doctors Weapons* (McGraw-Hill, NY, 456pp, $3). EH Hume describes LR Hubbard's "Dianetics" (*Look* Dec 5/50, p79). GS Perry does Galveston (*SatEvePost* Nov 25/50, p24). J Roddy laughs at DJ LeBlanc's "Hadacol" (*Look* Dec 5/50, p35). G Roheim offers *Psycho-Analysis & Anthropology* (Internat Univ Press, NY, 50, 496pp, $10). B Schulberg does Scott Fitzgerald in *The Disenchanted* (Random, NY, 50, 388pp, $3.5).

THE CLINIC: HM Adam & Co show high urinary excretion of histamine in urticaria (*Quart J Exp Physiol* 36/49/50). K Aterman claims cortisone inhibits fibrosis (*Lancet* 2/517/Nov 11/50). A Campbell & E Robertson report hexamethonium Br (3 gms daily) useful in hypertension (*Brit Med J* 2/804/Oct 7/50). RA Carey & Co sympose on ACTH & cortisone (*Bull Johns Hopkins Hosp* 87/349–509/50). FR Cherrill notes effects of disease on finger prints (*Nature* 166/581/Oct 7/50). DAW Edwards discusses distribution of subcutaneous fat (*Clin Sci* 9/259/50). GV Hall describes pathogenesis of atheroma (*Med J Austral* 2/475/Sept 23/50). FG Hirsch, EC Texter & Co describe red cell counting by electrical conductivity method (*Blood* 5/1017, 1036/50). DA Karnofsky reviews use of nitrogen mustards in neoplastic disease (*Adv Int Med* 4/1/50—NB edited by W Dock & I Snapper, these are excellent). DHK Lee discusses pathophysiology of tropical stress (*New Eng J Med* 243/723/Nov 9/50). GK Mallory & Co sympose on amniotic fluid embolism (*ibid.* p583, Oct 19/50). RP Osborne & JP Reidy discuss management of burns (*Brit Med J* 2/1025, 1030/Nov 4/50). B Robinson & Co survey sarcoidosis (*Med J Austral* 2/568/Oct 14/50). RB Thompson & LG Laitha report erythropoietic inhibiting factor in pernicious anemia (*Clin Sci* 9/281, 287/50). CS Welch reviews portal hypertension (*New Eng J Med* 243/598/Oct 19/50).

THE LAB: CB Albrecht notes adaptation to ingesting sea water & LD_{50} as 16% body wt on single dose (*Am J Physiol* 163/370/50). E Allara discusses structural relations of basement membrane (*Arch Ital Anat Embry* 55/163/50). G Asboe-Hansen says mast cells secreting hyaluronic acid transmit humoral activity to connective tissue (*Acta Dermat-Ven* 30/338/50). J Awapara & Co find aminobutyric acid in brain from glutamic acid (*J Biol Chem* 187/35, 267/50). LG Barth translates J Brachet's *Chemical Embryology* (Interscience NY, 50, 547pp, $8). S Bazin & R Robineaux say electrophoretic mobility of young white cells is higher than for old (*Compt Rend Soc Biol* 144/959/50). HT Blumenthal & Co emphasize interrelation of elastic tissue & calcium in arteriosclerosis (*Am J Path* 26/989/50). JT Edsall reviews plasma & clotting protein clinistry (*Ergeb Physiol* 46/308, 354/50). U. von Euler reviews noradrenalin as chemical transmitter of adrenergic nerves (*ibid* p261). HM Evans & Co show growth hormone survives 9 hrs in circulation &

Acth 17 minutes (*Am J Physiol* 163/297/50). CA Finch & Co discuss pathophysiology of iron storage (*Blood* 5/983/50). GW Harris & Co find emotional stress lymphopenia is rapid indicator of ant pituitary action & Acth is secreted by neural control via hypothalamus (*J Physiol* 111/328, 335/50). D Hill & G Parr edit *Electroencephalography* (Macmillan, NY, 50, 438pp, $13). P Holtz & HJ Schumann show relation of carotid sinus to spleen contraction (*Arch Exp Path Pharmakol* 211/1/50). H Hoseman considers infant size in relation to infant mortality (*Naturwis* 37/409/50). S Loewe reviews pharmacology of cannabis & cannabinol (*Arch Exp Path Pharmakol* 211/175/50). F Linder & J Vollman find 10 mgm "cliradon" (1-methyl-4-oxyphenyl-peperidin-4-ethylketone) safe analgesic with less resp depression than morphine (*Klin Wchnschr* 28/675/Oct 15/50). WFHM Mommaerts offers *Muscular Contraction* (Interscience NY, 50, 202pp, $4.2). ID Morton & AR Todd identify hemolytic agent fr horse brain as octadec-11-enoic acid (*Biochem J* 47/327/50). A Nilzen & H Paldrok note histaminolytic action of fungi (*Acta Dermat-Ven* 30/348/50). JR Pappenheimer's Committee recommends standards & symbols for respiratory physiology (*Fed Proc* 9/602/50). VR Potter & C Heidelberger discuss alternative metabolic paths (*Physiol Rev* 30/487/50). EM Trautner & IAN McCallum indicate tropines act as substituted acetylcholines by competitive fixation at receptors (*Austral J Exp Biol Med Sci* 28/343/50).

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